Memoirs

(1)

3(

MEMOIRS
OF THE
FACULTY OF FISHERIES HOKKAIDO UNIVERSITY

Vol. 3, No. 1

THE FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
1955

STUDIES ON THE PROTEINS OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

EIICHI TANIKAWA

Faculty of Fisheries, Hokkaido University

STUDIES ON THE PROTEINS OF THE MEAT OF SEA CUCUMBER

(*STICHOPUS JAPONICUS* SELENKA)*

EIICHI TANIKAWA

Faculty of Fisheries, Hokkaido University

CONTENTS

Introduction 1
I. Histological studies on the meat of *Stichopus japonicus* 3
II. Solubility of the meat proteins in solvents which are employed for the fractionation of protein mixtures 6
III. Fractionation of proteins in the meat 9
IV. Nitrogen distribution and amino acid composition of the fractionated proteins and the whole proteins of the meat part and the skin part of *Stichopus japonicus* 11
V. Isoelectric points of meat proteins of *Stichopus japonicus* 15
VI. Electrophoretic studies of the fractionated proteins of the meat of *Stichopus japonicus* 19
VII. Phenomenon of the dissolution of proteins in the meat of *Stichopus japonicus* 22
VIII. The phenomenon of flow birefringence of the aqueous extract of *Stichopus japonicus* meat and the electrophoretical behaviour of the water extracted proteins 32
IX. Heat contraction of the meat of *Stichopus japonicus* 43
X. The amount of collagen in the meat of *Stichopus japonicus* 45
XI. Dehydration of the meat of *Stichopus japonicus* 48
XII. Acid coagulation of the meat of *Stichoups japonicus* 53
XIII. The swelling of the meat of *Stichopus japonicus* 55
XIV. Hydration of the meat and meat proteins of *Stichopus japonicus* 62
XV. Hydration of the meat of *Stichopus japonicus* in NaCl solution 66
XVI. Hydration and swelling of the meat of *Stichopus japonicus* which was boiled and dried to different moisture contents 69
XVII. On the mucoprotein in the meat of *Stichopus japonicus* 74
XVIII. General discussion 82
Summary 87
Literature cited 89

INTRODUCTION

The author[1] has conducted studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus*) from the stand point of the utilization and processing of the meat. The following conclusions have been reached: (1) The chemical

* This "Studies on the proteins of the meat of sea cucumber (*Stichopus japonicus* SELENKA) is the third part of "Chemical studies on the meat of sea cucumber (*Stichopus japonicus* SELENKA)).

components of the meat of *Stichopus japonicus* vary with seasons. (2) The forms of nitrogen found in the meat of *Stichopus japonicus* are almost the same as those of fish meat. (3) The amount of water content of the former is larger than that of the latter. (4) The amount of total nitrogen is very small. (5) The digestibility of *Stichopus japonicus* meat is inferior to that of fish meat. (6) The calorific value of the former is less than that of the latter. (7) Therefore the meat of *Stichopus japonicus* is not a food to provide a protein source, but is a useful tasty food.

The author[2] has also investigated the post-mortem chemical and bacteriological changes of the meat of *Stichopus japonicus* ; he has observed : (1) The type of the putrefaction of the meat. (2) Methods for estimating the freshness of the meat when it is being processed, and (3) The relation between the freshness at time of canning and the quality of the canned food.

In the course of the previous experiments on the meat of *Stichopus japonicus*, the following differences were observed between the meat of *Stichopus japonicus* and of fish meat : The meat of *Stichopus japonicus* is more autolytic than other fish meats, and water in the meat can be driven off, and the phenomena of flow birefringence appeared in the aqueous extract as observed in that of squid meat[3]. When the canned food of *Stichopus japonicus* is preserved for a long time, the meat will get out of shape in the container. Even though it may be boiled and dried, the meat is more hydratable than fish meat.

Stichopus japonicus belongs to Echinodermata, and is situated lower than the fish in zoological classification. Even if the meat of *Stichopus japonicus* has the same chemical components of protein as fish meat by chemical analysis, it is supposed that differences may exist in the internal structure of protein, *i.e.* in size and shape of protein, and the molecular cohesion owing to linkages in protein. In the present paper, the author undertakes to clarify the chemical and physical properties of the meat of *Stichopus japonicus* by a series of experiments.

ACKNOWLEDGEMENT

Before going further the author wishes to acknowledge his indebtedness to several persons for their supporting of his work.

Gratitude is first offered to staff members of the laboratory, Messrs. Minoru AKIBA, Terushige MOTOHIRO, Yutaka FUJII, Hirotoshi ISHIKO, Jiro YAMASHITA and Tetsuro WAKASA, who cooperated in this investigation.

To Dr. Masao MIGITA, Mr. Toyo-o TAKAHASHI, and other colaborators in the laboratory of Protein Chemistry in Tokai Regional Fisheries Research Institute, grateful acknowledgement is due for giving helpful suggestions and criticisms in regard of this work.

Thanks are offered to Asist. Prof. Eijiro NIIYAMA, Faculty of Fisheries, Hokkaido University, for his help in the field of histological investigation.

Furthermore, thanks are also due to President Harusada SUGINOME of Hokkaido University and Prof. Naomoto TAKASUGI, Faculty of Science, Hokkaido University, at Sapporo, for their kindness in reading and correcting of this manuscript.

Finally the author wishes also to express his gratitude to Prof. Shigeru MOTODA and Mr. Takashi SUGIYAMA, Faculty of Fisheries, Hokkaido University, for their aid in preparing figures and manuscript for publication. And the author thanks Dr. H. M. LANE who has kindly consented to read over the manuscript.

I. HISTOLOGICAL STUDIES ON THE MEAT OF *STICHOPUS JAPONICUS*

Studies on the nature and form of the tissue of the meat are the basis of the chemical studies on its protein. The author has attempted to ascertain the histological properties of the meat. The body system of *Stichopus japonicus* was explained in the first of these papers titled "Nutritive studies on the meat of *Stichopus japonicus*"[1]. Here, matters to which attention must be paid in taking sample in this experiment will be explained.

On the dorsal side of *Stichopus japonicus* there are ordinarily comparatively large papillae, in which pedicellus are contained. Pedicellus are connected with the radial canals shown as in Fig. 1–1. Body of *Stichopus japonicus* was split on the dorsal side and eviscerated. Radial canals exist lengthwise along the body axis between the mouth and the anus. There are two radial canals on the dorsal side and three radial canals on the ventral side. On the ventral side, there are many and small pedicellus, which can be easily differentiated from the papillae of dorsal side. Muscular tissue which crosses at a right angle with body axis is observed between the radial canals and body wall as seen in Fig. 1–1. The dorsal side exhibits various colors according to the places where they live. In many cases, the color is brown or darkish brown.

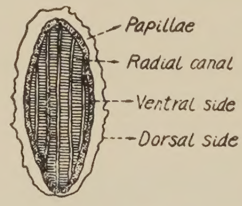


Fig. 1–1. Body of *Stichopus japonicus* was split on the dorsal side and eviscerated

(1) Position of samples taken and preparation of sample for microscopic examination

In this experiment, the body of *Stichopus japonicus* was divided into four parts : (A) middle layer of body wall, (B) upper layer of body wall, (C) body wall of coelum, containing radial canal, (D) upper layer of ventral side as shown in Fig. 1–2 (i). Slices (about $0.5 \times 0.5 \times 1.5\ cm^3$) were prepared from each part as above stated. From the part of middle layer of the body wall (A), slices were taken from side parallel with dorsal side (a), longitudinal side near coelum (b) and side of cross section (c) as indicated by shaded portions in Fig. 1–2 (ii). In the parts of (B) and (C), only one sample each from longitudinal section was taken ; in the part of (D), a slice from the side of the inner wall of coelum. All slices were microscopically observed. It is known that there

are many small calcareous pieces in the surface of skin part, therefore, the part of upper layer (B) was dehydrated by alcohol, skinned as thin as possible, and observed under microscope.

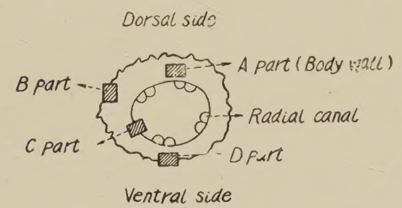


Fig. 1-2 (i) Sampling part

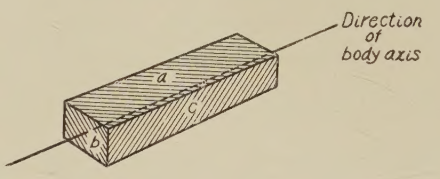


Fig. 1-2 (ii) Microscopically observed position

The slices of the tissues were fixed in Bouin's solution (saturated picric acid 75, formalin 25, acetic acid 5) for about two hours. The dehydration of the slices was done by alcohol of 70, 80, 90, 95% in order for 30 minutes each respectively. Then the slices were treated with absolute alcohol, and a mixed solution of creosote and toluene (1 : 1) for one hour respectively. Next the slices were treated with toluene for 30 minutes and with a mixed solution of toluene and paraffin (5 : 5) for one hour. Fixed slices thus obtained were cut in 10μ (or 15 μ) thick by microtome. For staining, each slice spread on the slide glass was fixed by drying, and then treated with benzine, absolute alcohol, 90, 70, 50% alcohol and water in order for 5 minutes each respectively. They were dyed with Delafield Haemotoxylin solution for 90 minutes and then washed in running water for 20 minutes. Next, each dyed slice was decolored moderately by acid-alcohol, then immersed in eosin alcohol solution for one minute, washed with 70, 90, 94% and absolute alcohol in order respectively, treated with a mixture of creosote and xylene (2 : 8) and with xylene and at last immersed in balsam. Thus the permanent specimen was made.


Fig. 1-3. Section of the middle layer of the body wall (×60)

(2) Microscopic observation

Fig. 1-3 shows the section (×60) of the middle layer of the body wall (A), Fig. 1-4 shows the section (×60) from part (C) which is between the body wall of coelum and radial canal. Fig. 1-5 shows calcareous pieces in the skin part.

As seen in Fig. 1-3 small black points which have been not dyed by eosin solution were nuclei which were dyed with haematoxylin. Such nuclei exist in the fibrous tissue. The directions in which fibrous tissues extend are irregular. This fact is observed also in the slices of parts of A, B, C and D.



Fig. 1–4. Section of the C part which is between the body wall of coelum and radial canal (×60)

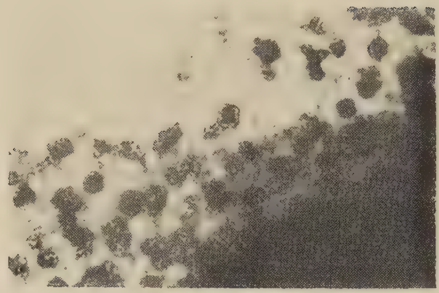


Fig. 1–5. Calcareous pieces in the skin part (×60)

The construction of the fibrous tissue is that of a network; each fiber is about 2–6μ thick. From the fact that the fiber is not dyed with eosin, the part of body wall is supposed histologically to be composed of collagen fiber, connective tissue, in greater part. Elastin fiber has not been found.

As seen in Fig. 1–4, both the tissues (M) point of radial canal and (N) which exists between (M) and (S) point of the body wall were clearly dyed with eosin solution, so that those tissues seem histologically to be muscular tissue; the thickness of muscular tissue fiber is about 1 μ. The thin fibrous tissue runs along the body axis at (M) point of radial canal, and runs across the body axis at (N) point of the tissue.

As seen in Fig. 1–5, there are many calcareous pieces (dia. about 25 μ) in the skin. Those pieces have various shapes according to the species of Holothurioidea. In part (D), the pedicellus pass from the skin to the coelum. In the slice which was obtained by cutting at right angles with pedicellus, the pedicellus exhibit a vacuous tube as seen in Fig. 1–6. The wall surrounding the pedicellus is thin muscular tissue, and the outer part of the muscular tissue appears to be a blending fiber tissue of the connective tissue.

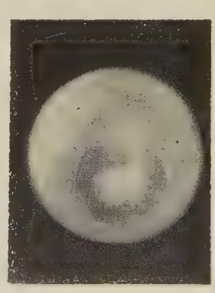


Fig. 1–6. Pedicellus with vacuous tube (×75)

From the histological observation, the meat of *Stichopus japonicus*, edible part, is seen to be not true muscular tissue, but connective tissue. The true muscular tissue comprises the radial canals, the tissue which links the radial canals with body wall, and the tissue surrounding the anus. The main edible part of *Stichopus japonicus* is connective tissue as above stated. Among the network of the connective tissue, there seems to be contained a large amount of water.

The author has studied the proteins of the edible part of *Stichopus japonicus* which is called "meat" to conform to custom. In the following experiments, the author has used the expression "so-called meat" or "meat" for the

edible part (A-part) of the body wall of *Stichopus japonicus*. The skin part is also the part (B) as shown in Fig. 1–2 (i) containing the derma.

II. SOLUBILITY OF THE MEAT PROTEINS BY SOLVENTS WHICH ARE EMPLOYED FOR THE FRACTIONATION OF PROTEIN MIXTURES

It is important to know the adequate concentration and volume of solvents for the fractionation of protein mixtures. The author has tried to determine the concentration and the volume of NaCl and NaOH solutions and the volume of water which are used for the fractionation of proteins of the meat of *Stichopus japonicus*.

(1) Sample

Bodies of *Stichopus japonicus* which were caught in the sea near Hakodate in May, were divided into two parts, meat part and skin part, as shown in Fig. 1–2 (i). Only the meat part was employed. The amount of water content and total nitrogen of the meat part are shown as Table 2–1. In this study, as the number of experiments run was large, and the bodies of *Stichopus japonicus* which were caught on the same day were insufficient, it was necessary to employ bodies which were caught within a period of several days.

Table 2–1. The amount of water content and total nitrogen of *Stichopus japonicus* meat.

Sample	Water content in %	Total nitrogen in %
I	88.75%	1.35%
II	88.75%	1.36%

(2) In the case of employing water as the solvent

(i) The amounts of protein dissolved in various length of extraction time

To 5 g of the meat was added ten times its volume of water; material was left at room temperature. The extracts were filtered by filter paper at the interval of the definite time. Ten cc of the filtrate was used for the estimation of water soluble total nitrogen. To ten cc of the filtrate was added 10% trichloracetic acid, whereupon proteins were precipitated. After the filtration of proteins, the precipitated protein nitrogen was estimated by usual method. The results obtained are shown in Fig. 2–1.

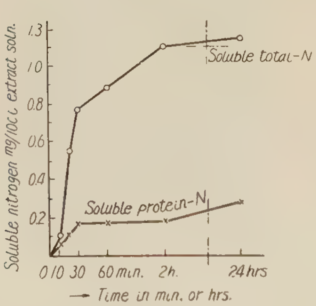


Fig. 2–1. The amount of dissolved nitrogen in water (Soluble-N mg/10 cc extracted solution)

As seen in Fig. 2–1, the amount of water soluble total nitrogen is only 130 mg% for the raw meat, and the amount of the protein nitrogen is about 23% of the total water soluble nitrogen. In samples which had been extracted by water for periods up to 30

minutes, the amount of water soluble total nitrogen increased rapidly, but after 24 hours extraction the amounts are almost constant. Accordingly, the author has determined that the suitable extraction time is 24 hours.

(ii) The amounts of proteins dissolved by the use of different volumes of water in the extraction

The amounts of water which are added to the meat were varied between 10 cc and 400 cc; the amounts of dissolved protein nitrogen were estimated. That is to say, 10, 20, 30, 50, 100, 200, 300, 400 cc of water were added to each 10 g of the meat respectively and the mixture were left for 24 hours at room temperature after 30 minutes shaking. After that period of standing, each mixture was filtered. To each 10 cc of the filtrate was added 10% trichloracetic acid (but in the case of addition of 10 cc of water to 10 g of the meat, 5 cc of the filtrate was employed). The amount of precipitated protein nitrogen in each mixture was estimated by usual method. The results obtained are shown in Fig. 2–2.

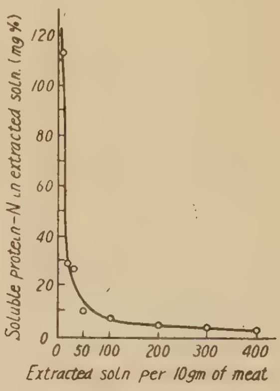


Fig. 2-2. The amounts of protein nitrogen dissolved in various volumes of water

As seen in Fig. 2–2 the smaller the amount of the solvent (water) is, the greater the solubility of the meat proteins in the filtrate becomes. But the total amount of protein nitrogen in the total volume of the solvent is not necessarily as large for the smaller amount of the solvent. If greater solubility of the meat proteins and easy filtration are desired, 50 cc of water should be added for 10 g of the crushed meat. The author has employed this prescription for the fractionation of water soluble protein.

(3) In the case of employing NaCl solution as the solvent

(i) The amounts of proteins dissolved by various concentrations of NaCl solution

In order to ascertain the optimum concentration of NaCl solutions for the extraction of proteins in the meat, 0.2~3.0 Mol of NaCl solutions of 10 times the volume of 10 g of the meat was added respectively, and each mixture was left for 24 hours at room temperature after 30 minutes shaking. After the standing time, the mixture was filtered. Ten cc sample of the filtrate were used for the estimation of the amounts of protein nitrogen. To each 10 cc sample of the filtrate was added 10% trichloracetic acid solution and protein was precipitated. The amount of protein nitrogen was estimated. The results obtained are shown in Fig. 2–3.

As seen in Fig. 2–3, the amount of NaCl solution-soluble total nitrogen was greatest in 1 Mol of NaCl solution. But for the sake of convenience in treatment, 0.6 Mol NaCl solution was used for the extraction of NaCl solution-soluble proteins.

(ii) The amounts of protein nitrogen dissolved in the various volumes of NaCl solution used for the extraction

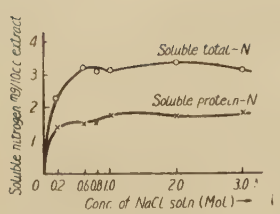


Fig. 2-3. The amounts of protein nitrogen dissolved in various concentrations of NaCl solutions

The amount of 0.6 Mol NaCl solution which was added to the meat was varied between 10 cc and 400 cc; the amounts of dissolved protein nitrogen were estimated. That is to say, 10, 20, 30, 50, 100, 200, 300, 400 cc of 0.6 Mol NaCl solution were added respectively to each 10 g sample of the meat and the mixtures were left for 24 hours at room temperature after 30 minutes shaking. After the standing time, each mixture was filtered. To each 10 cc of the filtrate was added 10% trichloracetic acid solution, and the amount of protein nitrogen was estimated by usual method. The results obtained are shown in Fig. 2–4.

As seen in Fig. 2–4, the optimum volume of 0.6 Mol NaCl solution was considered to be 50 cc for 10 g of the crushed meat for the best extraction of NaCl solution-soluble proteins.

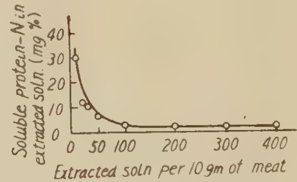


Fig. 2-4. The amounts of protein nitrogen dissolved in various volumes of 0.4 Mol NaCl solution

(4) In the case of employing NaOH solution as the solvent

(i) The amounts of proteins dissolvsd by various concentrations and volumes of NaOH solution

In order to learn the optimum concentration and volume of NaOH solution for the extraction of proteins in the meat, the same method as in the previous experiment was employed. From 0.01 to 0.1 Mol of NaOH solutions of 5~20 times the volume of 10 g of the meat were used respectively, and the amounts of NaOH solution-soluble total nitrogen and protein nitrogen were estimated. The results obtained are shown in Fig. 2–5.

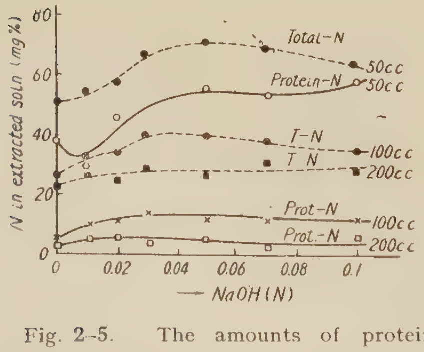


Fig. 2-5. The amounts of protein nitrogen dissolved in various concentrations and volumes of NaOH solutions

As seen in Fig. 2–5, the amounts of NaOH solution-soluble protein nitrogen reached its maximum in 50 cc of 0.05 Mol NaOH solution for 10 g of the meat. But for the sake of convenient treatment, 0.025 Mol NaOH solution is satisfactory. For the extraction of NaOH solution-soluble protein, 100 cc of 0.025 Mol NaOH solution was used for 10 g of the crushed meat.

III. FRACTIONATION OF PROTEINS IN THE MEAT

From the fundamental experimental results as previously obtained, the fractionation of water soluble proteins, NaCl solution soluble proteins and dil. NaOH solution-soluble proteins was carried out. The bodies of *Stichopus japonicus* caught in the sea near Hakodate in September were divided into two parts, meat part and skin part. Fractionated proteins were prepared from only the crushed meat.

(1) Fractionation of water soluble proteins

To one kg of fresh crushed meat was added 5 *l* of water ; the mixture was shaken for 3.5 hours and then left in an ice-box for 24 hours. After the end of that period, the mixture was centrifuged (4,000 r.p.m.) for 30 minutes. The sediment was once more diluted with 2 *l* of water and was shaken for about 1.5 hours, then centrifuged. The clear centrifuged liquor was gathered. This is water soluble protein solution. Seven *l* of the extract (light brown liquid) was added with ammonium sulfate with gentle shaking. When the liquor was half saturated, a light brown sediment was obtained. After that standing for 2 days, the sediment was centrifuged. The upper clear liquor obtained was 2/3 saturated ; it was left for 2 days, but there was no sediment. The separated sediment was dialyzed for 7 days, and then washed with alcohol and ether in order, and dried in a vacuum desiccator. Thus, about 3.5 g of the fraction of water soluble proteins was obtained (the yield was about 0.35%). In order to ascertain the existence of any remaining protein, the upper clear liquor obtained by the centrifugation was saturated with ammonium sulfate and the pH of the solution was adjusted to 5.2, but no sediment was formed. The same clear liquor above obtained was also negative upon the addition of trichloracetic acid.

(2) Fractionation of NaCl solution-soluble proteins

To the residue of the meat insoluble by water was added 5 *l* of 0.6 Mol NaCl solution. The mixture was left for 24 hours after 2 hours' shaking. After the end of that period, the mixture was centrifuged (4,000 r.p.m.) for 30 minutes and the upper liquor was filtered again. The sediment was washed with alcohol and ether in order, and then dried in a desiccator. As a result, about 5 g of the fraction of NaCl solution-soluble proteins was obtained (the yield was about 0.5%).

(3) Fractionation of dil. NaOH solution-soluble proteins

To the residue (about 900 g) of the meat insoluble by NaCl solution was added 5 *l* of 0.025 Mol NaOH solution. The mixture was left for 2 days after 2 hours' shaking. After that standing, the mixture was centrifuged. The upper clear liquor was adjusted to pH 4.5 by addition of 2 Mol CH_3COOH solution and left for one day, then a white

sediment was formed. This sediment was divided into two parts, 2/3 and 1/3 parts. The former part was dialyzed for 7 days, and then centrifuged. The sediment was washed with alcohol and ether in order and then was dried. This is called the dil. NaOH solution-soluble protein I. The amount was about 5 g (yield is about 0.5%). The latter part was dissolved with 200 cc of 0.025 Mol NaOH and was adjusted to pH 4.4 by addition of 2 Mol CH_3COOH solution. The sediment formed was separated and then dialyzed for 7 days, centrifuged, washed with alcohol and ether in order and dried in a desiccator. This is the dil. NaOH solution-soluble protein II, and the amount was about 3 g (the yield was about 0.3%).

From the above-described treatments, about 10% of water soluble proteins, about 12% of NaCl solution-soluble proteins, and about 1% of the dil. NaOH solution-soluble proteins for the total amount of proteins, respectively were obtained.

(4) Preparation of whole protein of the meat

In order to find out the nitrogen distribution of the whole protein of the meat part and skin part, the whole protein was prepared from the meat part and skin part according to the following treatments.

The bodies were divided into two parts, meat part and skin part. Each part was crushed with a sharp knife. To one hundred g of the two curshed parts respectively were added with 300 cc of 94% alcohol. Each mixture was heated on a water bath for one hour. The upper clear liquor was decanted. The residue was crushed again, and to it was added 300 cc of 94% alcohol. Such treatment was repeated three times. The fat in the residue was removed twice with ether. The alcohol and ether in the residue were removed on a water bath ; the residue was dried in a desiccator and then crushed. In the first extraction from the meat and skin parts by alcohol, there is dissolved a part of the water soluble proteins. Therefore, to the upper clear liquor of the extraction was added again twice the volume of 94% alcohol previously. Then

Table 3-1. The yield and chemical components of fractionated proteins and the whole protein of the meat and the skin part of *Stichopus japonicus*

Fractionated proteins		Yield (in raw sample)	Per cent for the amount whole protein prepared from the meat part	water content	in dry matter	
					nitrogen	ash
Water Soluble Proteins		0.35%	5.22%	25.5%	14.2%	5.7%
0.6M. NaCl Soln. Soluble Proteins		0.5	7.47	32.1	13.5	7.5
0.025M. NaOH Soln.-Soluble Proteins	I	0.5	7.47	26.3	14.8	4.8
	II	0.3	4.48	22.4	14.1	—
Whole protein of the meat part.		6.7	100	21.8	12.7	72.8
Whole protein of the skin part.		5.2	—	24.8	13.2	18.0

the proteins were precipitated. The precipitated proteins were mixed with the residue previously obtained. This mixture was regarded to be the whole protein. The whole protein of the meat part was a grayish white and that of the skin part was a grayish dark powder. The yields and chemical components of the fractionated proteins and the whole protein of the meat part and skin part are shown in Table 3–1.

As seen in Table 3–1, the amount of nitrogen content in the fractionated proteins of the meat of *Stichopus japonicus* comes to 13~15%. That amount of nitrogen content is less than that of fish meat which is about 16%. However, the amounts of ash content in the whole proteins of the meat part and skin part of *Stichopus japonicus* are comparatively large, 13~18%. The fact that the amount of ash content in the whole protein of the skin part is especially large is perhaps principally due to the existence of calcareous pieces in the skin part as observed in the histological investigation (Article I). The ash is contained also in the water soluble protein, NaCl solution-soluble protein and dil. NaOH solution-soluble protein of the meat of *Stichopus japonicus* to the amount of about 5~7%. The presence of ash in the fractionated protein may be also due to the existence of calcareous pieces in the meat.

IV. NITROGEN DISTRIBUTION AND AMINO ACID COMPOSITION OF THE FRACTIONATED PROTEINS AND THE WHOLE PROTEIN OF THE MEAT PART AND THE SKIN PART OF *STICHOPUS JAPONICUS*

After the HCl-hydrolysis of five kinds of protein which were fractionated and prepared from the meat of *Stichopus japonicus* by the methods described in the previous Article, the nitrogen distribution of the hydrolysates was determined by Van Slyke's method[4]. Also amino acid compositions of the hydrolysates were determined by paper chromatography.

1. Nitrogen distribution and amino acid composition of the fractionated proteins

The results of the determination of nitrogen distribution of the water soluble protein, 0.6 Mol NaCl solution-soluble protein and 0.025 Mol NaCl solution-soluble protein I are shown in Table 4–1. For comparison, the results with *Stichopus japonicus* and those with the meat of Nibe-fish (*Scioend albiflora*)[5] are shown in Table 4–2. In that table, the amount of 20% HCl solution-soluble nitrogen was taken as 100, and the percentages of the amount of nitrogen in various states are shown, respectively.

As seen in Table 4–1, about 20% of the total nitrogen of various proteins is basic amino acid nitrogen, and 55~60% is monoamino acid nitrogen. The amount of basic amino acid nitrogen is the largest in water soluble protein, with those of dil. NaOH solution-soluble protein and NaCl solution-soluble protein coming next, but there is no great difference among them. The order of decrease in the amount of monoamino acid nitrogen is similar with that of the basic amino acid nitrogen. There is also no

great difference among the amounts of monoamino acid nitrogen.

Table 4-1. Nitrogen distribution of the fractionated proteins of *Stichopus japonicus* meat

Fraction	Water solube protein		0.6M NaCl soluble protein		0.025M NaOH soluble protein	
	in sample (%)	in total-N (%)	in sample (%)	in total-N (%)	in sample (%)	in total-N (%)
Total-N	8.208	100	7.029	100	8.028	100
20% HCl insoluble-N	0.348	4.24	0.335	4.77	0.289	3.60
20% HCl soluble-N	7.981	97.24	6.652	94.6	7.455	92.81
Amide-N	0.517	6.30	0.758	10.78	0.570	7.10
Humine-N	0.499	6.08	0.671	9.55	0.488	6.08
Total-N in form of bases	1.900	23.15	1.392	19.85	1.753	21.84
Arginine-N	1.099	13.39	0.828	11.18	1.049	13.07
Histidine-N	0.365	4.45	0.208	2.87	0.196	2.44
Lysine-N	0.388	4.73	0.308	4.30	0.428	5.83
Cystine-N	0.048	0.58	0.062	0.95	0.079	1.00
Amino-N in form of bases	0.832	10.14	0.639	9.09	0.835	10.40
Total-N in form of monoamino fraction	4.829	58.83	3.877	5.16	4.371	56.94
Amino-N in form of m.a. fraction	4.199	51.04	3.508	49.91	4.045	50.38
Non-amino-N in form of m.a. fraction	0.630	7.79	0.369	5.25	0.526	6.56
Sum	8.093	98.60	7.036	100.09	7.671	95.55
Water content in %	40.8%		45.2%		43.2%	

Table 4-2. Comparative values of the nitrogen distribution of *Stichopus japonicus* meat and that of the meat of "Nibe-fish"

Fraction	Protein of Nibe-fish meat			Proteins of *Stichopus japonicus* meat		
	Water soluble protein	0.6M NaCl soluble protein	0.025M NaOH soluble protein	Water soluble protein	0.6M NaCl soluble protein	0.025M NaOH soluble protein
20% HCl soluble-N	100	100	100	100	100	100
Amide-N	9.53	7.27	7.54	6.48	11.39	7.65
Humine-N	8.63	6.09	6.16	6.25	10.09	6.55
Total-N in form of bases	16.69	25.52	33.17	23.81	20.99	23.52
Arginine-N	6.39	7.2	9.17	13.77	12.45	14.07
Histidine-N	6.49	11.50	15.49	4.58	3.03	2.63
Lysine-N	6.68	6.62	8.32	4.86	4.54	5.74
Cystine-N	0.16	0.18	0.29	0.60	1.00	1.07
Amino-N in form of bases	10.59	12.44	15.98	10.83	9.60	11.20
Total-N in form of mono amino fraction	63.27	59.61	51.83	60.50	58.28	61.32
Amino-N in form of m.a. fraction	60.92	38.43	49.85	52.53	52.74	54.25
Non-amino-N in form of m.a. fraction	2.35	1.16	1.98	7.97	5.54	7.07
Sum	98.12	98.48	98.69	97.04	100.73	99.04

Numerals are per cent for 20% HCl soluble-N.

The amounts of amide nitrogen and humine nitrogen are the greatest in NaCl solution-soluble protein and are smallest in water soluble protein. The amount of arginine nitrogen is 2 to 5 times the amount of each other amino acid nitrogen in the basic amino acid nitrogen. It may be considered that there is some quantitative difference in the nitrogen distribution of proteins, because even the fracticonated protein is not unique, but is considered to consist of a few components of protein[6]. Next, from Table 4–2, comparing the nitrogen distribution of the fractionated proteins of *Stichopus japonicus* with that of Nibe-fish meat, the amounts of amide nitrogen and humine nitrogen of the former are larger than the latter in NaCl solution-soluble protein, but they are the opposite in water soluble protein. There are remarkable differences in the amount of basic amino acid nitrogen in water soluble protein and dil. NaOH solution-soluble protein between *Stichopus japonicus* and Nibe-fish. For example, the amount of arginine nitrogen of the meat of the former is remarkably larger than that of the latter. On the contrary, the amount of histidine nitrogen of the former is fairly smaller than that of the latter. There is slight difference of the amount of lysine nitrogen between the two. The amount of cystine nitrogen of the former is smaller than that of the latter. The amount of monoamino acid nitrogen in the dil. NaOH solution-soluble protein of *Stichopus japonicus* is larger than in that of Nibe-fish. But there is no difference in water soluble protein and in NaCl solution-soluble protein. From the results as above obtained, the nitrogen distribution of the proteins of the meat of *Stichopus japonicus* is considered to be almost the same as that of proteins of flat fish, seabream, shark and Nibe-fish meat.

Next, the HCl-hydrolysate of each fractionated protein was used for two-dimensional paper chromatography. The developing reagents were phenol with 10% water, and a mixture solution of lutidine, anilins and water (65 : 7 : 28) ; the revealing reagent was ninhydrin-butanol solution. Revealed amino acids were identified. In water soluble protein of the meat of *Stichopus japonicus*, aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, proline, hydroxyproline, serine, arginine, lysine, ornithine, cystine, threonine, 3,5–dijodotyrosine, tyrosine, leucine, glutamine, methionine, and histidine were identified. The kinds of amino acids identified in NaCl solution-soluble protein and dil. NaOH solution-soluble protein were almost the same as those of water soluble protein. Consequently, the kinds of amino acids which compose the meat proteins of *Stichopus japonicus* are almost the same as those of fish meat.

2. Nitrogen distribution of the whole protein of the meat part and the skin part of *Stichopus japonicus*

Results of investigation of the nitrogen distribution of the whole protein of the meat part and the skin part which were obtained by the same method as previous described are shown in Table 4–3.

Table 4–3. Nitrogen distribution of the whole protein prepared from the meat and skin parts of *Stichopus japonicus*

Fraction	Whole protein of the meat part		Whole protein of the skin part	
	in sample (%)	in total-N (%)	in sample (%)	in total-N (%)
Total-N	9.926	100	9.934	100
20% HCl insoluble-N	0.368	3.71	0.291	2.93
20% HCl soluble-N	9.500	95.71	9.579	96·43
Amide-N	0.517	5.21	0.771	7.76
Humine-N	0.368	3.71	0.361	3.63
Total-N in form of bases	2.000	20.21	1.992	20.05
Arginine-N	0.868	8.75	1.097	11.04
Histidine-N	0.597	6.02	0.343	3.45
Lysine-N	0.440	4.43	0.475	4.78
Cystine-N	0.095	0.96	0.076	0.77
Amino-N in form of bases	0.951	9.58	0.940	9.47
Total-N in form of mono-amino Fraction	6.107	61.52	6.126	61.67
Amino-N in form of m.a. fraction	5.250	52.89	5.203	52.38
Non-amino-N in form of m.a. fraction	0.857	8.63	0.923	9.29
Sum	9.361	94.31	9.541	96.04
Water content in %	23.0%		21.8%	

As seen in Table 4–3, the amounts of the total nitrogen in each 100 g of the whole proteins of the meat part and the skin part are almost equal. The amount of 20% HCl insoluble nitrogen of the whole protein of the meat part is slightly larger than the skin part. This may be due to the concentration of the hydrolyzing agent and to the hydrolyzing time, so it seems to be insignificant. The amount of amide nitrogen in the whole protein of the meat part is smaller than that of the skin part. The amount of humine nitrogen of the whole protein of the meat or skin part is smaller than that of raw meat or the fractionated protein. The reason for this difference is not clear. The amount of total nitrogen of basic amino acid is about 20% of the amount of total nitrogen in both parts. The amount of arginine nitrogen is the largest in the basic amino acids. The amount of lysine nitrogen is about 4.5% in both the parts with no difference in the two parts. The amount of histidine nitrogen of the meat part is 6% and that of skin part is 3.5%. The amount of monoamino acid nitrogen of the two parts, meat and skin, is about 62%, which is almost equal to the nitrogen distribution of each fractionated protein. From the above stated data, it is considered that the nitrogen distribution of the whole protein of the meat and the skin parts is almost equal to the fractionated protein, except that the amount of humine nitrogen in the former is smaller than that in the latter.

To generalize regarding nitrogen distribution and amino acid composition of the fractionated proteins and the whole proteins, if the fact that the amount of protein content of the meat of *Stichopus japonicus* is very small (about 1% of the amount of

total nitrogen) is disregarded, the chemical components of protein of the meat show almost no difference from those of fish meat, but there seem to be remarkable differences in the construction of the protein of the meat of *Stichopus japonicus* from that of fish meat, as seen from the histological observations.

V. ISOELECTRIC POINT OF MEAT PROTEINS OF *STICHOPUS JAPONICUS*

In order to learn further about the characteristics of the meat proteins of *Stichopus japonicus*, the isoelectric points of the fractionated proteins from the meat, *viz.*, water soluble protein, NaCl solution-soluble protein and dil. NaOH solution-soluble protein I, were determined by the viscosity method and by the estimation of dissolved nitrogen of the meat extracts which were extracted from the meat immersed for definite lengths of time in various pH solutions.

1. Isoelectric points of the fractionated proteins

(1) Experimental method

A definite quantity of each fractionated protein was weighed, diluted with some quantity of dist. water, mixed up and then was left to allow the protein to inflate for 24 hours at room temperature. The swollen protein was transferred into a measuring flask with appropriate volume of dist. water; 60 cc of 0.1 Mol NaOH solution was added and then dist. water to bring the total volume up to 250 cc. The protein solution thus obtained was left again for 24 hours at room temperature, and was filtered through pulp layer. The filtrate was used as a sample. Ten cc of each solution of various kinds of protein was poured into a bottle of 100 cc volume, and buffer solution of various pH was added with dist. water to reach 50 cc in total volume. After leaving for a definite time, the precipitated substance was filtered. The pH value of the filtrate was determined by a glass electrode meter. The viscosity of the filtrate was measured by Ostwald's viscosimeter. The relative viscosity was calculated from those viscosity values. The pH value of the solution containing protein which indicates the minimum relative viscosity is regarded as the isoelectric point of the protein. In the case of dil. NaOH solution-soluble protein I, the total amount of nitrogen of the precipitated protein in 10 cc of the filtrate having various pH values was estimated. Comparing the isoelectric points which were determined by the viscosity method or by the method of the estimation of the total amount of nitrogen in the precipitated protein, the two isoelectric points were in agreement, therefore the total amount of nitrogen in the other proteins was not estimated.

(2) Experimental results

The results obtained are shown in Fig. 5–1∼Fig. 5–3. Here, the abscissa in the Figs. indicates the pH value of the filtrate.

As seen in each of the Figs., the isoelectric point of water soluble protein is pH

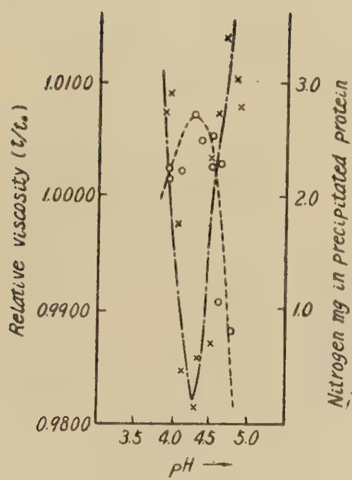


Fig. 5-1.

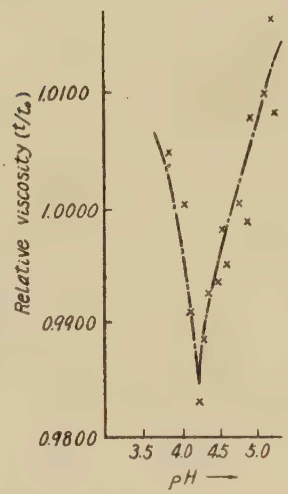


Fig. 5-2.

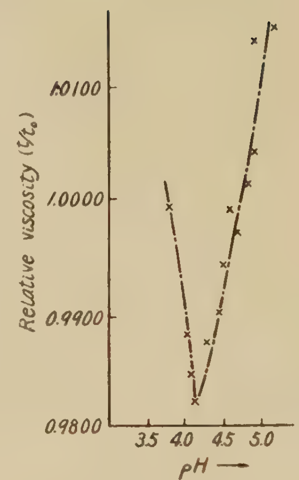


Fig. 5-3

Fig. 5-1. Isoelectric point of 0.025 Mol NaOH soluble protein
Fig. 5-2. Isoelectric point of water soluble protein
Fig. 5-3. Isoelectric point of 0.6 Mol NaCl soluble protein

4.2, NaCl solution-soluble protein is 4.13, dil. NaOH solution-soluble protein is 4.26.

2. Isoelectric point of the meat of *Stichopus japonicus*

(1) Experimental method

To 5 cc the crushed meat of *Stichopus japonicus*, 50 cc of the buffer solution of various pH values was added, and the mixture was left in an air-tight vessel over night at 10°C ± 2°C; it was then filtered. The pH value and the total nitrogen in 10 cc of the filtrate were estimated. The viscosity was measured by Ostwald's viscosimeter in the water vessel at constant temperature of 25°C ± 1°C. The adjustment of pH was made by the addition of 0.2 Mol CH_3COOH and 0.1 Mol NaOH solution. The solutions of pH 2.6 and pH 2.8 were adjusted by means of 0.01 Mol HCl and 0.2 Mol CH_3COOH.

(2) Experimental results

Results obtained are shown in Fig. 5-4. The abscissa of Fig. 5-4 indicates the pH value of extracted filtrate. Here, the difference of components of the buffer solutions was negligible. As may be noted from Fig. 5-4, the relative viscosity and the total amount of dissolved nitrogen of the extracts from the meat of *Stichopus japonicus* show minimum at pH 4.2. Accordingly, the approximate isoelectric point of the meat is considered to be pH 4.2. From the results as above stated, the isoelectric point of meat protein of *Stichopus japonicus* is considered to be about 4.2.

3 Discussion.

The isoelectric point of the meat of *Stichopus japonicus* is pH 4.2. As concluded by the use of similar methods, the isoelectric points of Akta mackerel meat was pH 5.4[7], and squid meat was pH 4.3[8]. According to the method of estimation by minimum degree of swelling of the meat, the isoelectric point of squid meat is pH 3.5–4.0 in contrast with pH 4.2 of carp meat (Noguchi[9]), and also squid meat is pH 4.8 in contrast with pH 5.5 of horse-mackerel meat (Okada[10]). The isoelectric point of squid meat is more on the acidic side than for fish meat. The isoelectric point of *Stichopus japonicus* is also more acidic than for fish meat. Migita[8] has raised questions in connection with the fact that the isoelectric point of squid meat is situated more on acidic side than most fish meat: (1) Squid meat may be predominant in the amount of acidic amino acids content, (2) It may have large amount of acidic components in the meat extract. However, after chemical examination directed toward a solution of those question, the composition of amino acids in the meat and extract of squid are ascertained to be almost the same as those of the fish meat. Therefore Migita has pointed out that the cause of the fact is not a difference of the compositions between squid meat and fish meat. Migita, next, has offered following questions; (3) squid meat may have acid charged radicals on the surface of the protein molecule, or (4) the ionic strength of the extract of squid meat may be larger than that of fish meat. According to Migita, question (4) originated from the statement of Hardy[11] that one feature of fundamental importance, which is never obscured .... is the antagonism between the solvent actions of salts and acids, and the additive nature of the combined solvent action of salt and alkali. That is to say, as a consequence of the antagonism between acid and salt, the value of pH, in which the solubility of the protein is at the minimum, slips down to the acidic side. If the ion strength of the extract of squid meat is larger than that of fish meat, the approximate isoelectric point of the meat protein of squid is considered to be more on the acidic side than that of fish meat protein. As to question (3), Ōtake[12] is now studying; according to him, the distribution of charged radical on the surface of the protein of squid meat is supposed experimentally to be one side of negatively charged group as compared with fish meat protein.

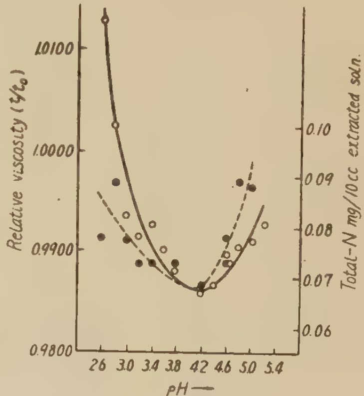


Fig. 5-4. Isoelectric reaction of aqueous extracted solution

Before undertaking a detailed examination of questions (3) and (4) regarding the

meat of *Stichopus japonicus*, the author has made estimate of the amount of acidic amino acids (glutamic acid, aspartic acid) of the fresh raw meat of *Stichopus japonicus* compared with that of Atka mackerel meat and squid meat. The estimation was carried out by Turba and Rechter's method[13]. A definite quantity of the crushed meat (100 g of the meat of *Stichopus japonicus*, each 50 g of the meat of Atka mackerel and squid were employed) was hydrolyzed by 6 Mol HCl solution for 15~20 hours. On the other hand, the adsorbing column of alumina was prepared from the activation by 1 N acetic acid buffer solution of pH 3.3. Acidic amino acids in the diluted hydrolysates of sample meat, which were developed respectively by N/10 acetic acid buffer solution (pH 3.3), were adsorbed by the activated alumina column. After the development, glutamic acid was dissolved out with 1 N acetic acid buffer solution of pH 3.3, and on the other hand, aspartic acid was dissolved out with N/20 NaOH solution. The amounts of amino acid nitrogen of glutamic acid and aspartic acid were estimated by Pope-Stevens' method[14].

Results obtained are shown in Table 5–1.

Table 5-1. Comparative amounts of acidic amino acid of the meat of *Stichopus japonicus* and those of the meat of Atka mackerel and squid

Sample	Glutamic acid		Aspartic acid	
	% in total protein	Glutamic Acid-N in total protein-N	% in total protein	Aspartic acid-N in total protein-N
		(%)		(%)
Stichopus japonicus	39.1	24	1.14	0.80
Atka mackerel	1.59	0.94	0.52	0.24
Squid	1.70	1.01	0.29	0.19

Note: Numerals in table are calculated as the amount of nitrogen content in glutamic and asparitc acids is 9.453 and 10.52 % respectively, and that in meat protein used are 16%.

As seen in Table 5–1, the meat of Atka mackerel and squid contain about 1.6 % of glutamic acid and about 0.3~0.5% of aspartic acid in the amount of total protein nitrogen content respectively. On the other hand, *Stichopus japonicus* meat contains about 39% glutamic acid and about 1% of aspartic acid in the total protein nitrogen content. That is to say, the latter possesses a larger amount of acidic amino acids than the former. Matsumoto[15] has also ascertained that squid and horse-mackerel meat contain about 13~15% of glutamic acid and about 8~10% of aspartic acid. There is room for the examination of the accuracy of the method of estimation employed in the fundamental experiment, *e.g.*, the amount of the added hydrolyzing reagent, the heating temperature, and the heating time, etc. Also it may be seen in Table 5–1, that the amount of aspartic acid is smaller than the amount of glutamic acid. This result agrees with Matsumoto's result in the difference between the two acids in the meat of squid and fish meat, and in the slight difference of the amount of acidic amino acids

between squid and fish meat. In the total amount of nitrogen of protein of *Stichopus japonicus* meat, the acidic amino acid content makes up 25%, *i.e.* glutamic acid is 24% and aspartic acid is 0.8%. This amount of acidic amino acid corresponds to about 53% of the monoamino acid nitrogen distribution as described in Article IV. Such a large amount of acidic amino acids can not be found in squid or horse-mackerel. The amount of basic amino acids is 20~30% of the total protein nitrogen of *Stichopus japonicus* meat as was stated above in Article IV, and this amount is almost equal to the corresponding value of Nibe-fish or Atka mackerel meat. The fact that the iso-electric point of *Stichopus japonicus* meat slips down to the acidic side is considered to be owing to the presence of the larger amount of acidic amino acids in the meat. Other causes which should be considered call for further examinations.

VI. ELECTROPHORETIC STUDIES ON THE FRACTIONATED PROTEINS OF THE MEAT OF *STICHOPUS JAPONICUS*

The uniformity of fractionated proteins was determined by means of electrophoretic examination .

1. Sample

To determine the concentration of proteins employed is the most important thing in this experiment. For a clear electrophoretic pattern, the most suitable concentration is 0.5~2%. It is difficult to dissolve the dried fractionated proteins, so the author dissolved the dialyzed sample immediately half-way in the preparation without treatment by alcohol and ether, and thus obtained fractionated proteins of the desired concéntration.

(1) Water soluble proteins

The water extract from the crushed meat was half saturated with ammonium sulfate. About 10 g of the sediment thus obtained was dialyzed against running water for one week (water temperature, 10°~12°C). After centrifugation, about 4 g of the sediment thus obtained was dissolved in phosphate buffer solution of which ionic strength, μ, was 0.15 and pH 8.1, and the total volume was made up to 200 cc. For fear some kind of protein might remain in the upper clear liquor after the centrifugation, the liquor was saturated with ammonium sulfate according to Subba Rao[16], and was adjusted to pH 5.4, but there was no sediment. To the liquor was also added 10% trichloracetic acid, but there was no sediment. The author has therefore considered that there is no protein in this part. After vigorous shaking, the sample solution was left at cool place over night, so almost all the sediment was dissolved. Ten cc of this sample solution was dialyzed in an ice-box against 500 cc of phosphate buffer solution having the same ionic strength. After 48 hours' dialysis, the sample solution was centrifuged. A clear light yellowish brown protein solution was obtained.

This solution was used for the electrophoresis. The solution was pH 6.74 and obtained about 0.3% of the concentration of the protein.

(2) NaCl solution-soluble proteins

Six-tenths Mol NaCl solution-soluble protein solution was saturated with ammonium sulfate. The sediment was dialyzed and was centrifuged in the same manner as above in **(1)**. About 4 g of the centrifuged sediment was dissolved in the same phosphate buffer solution, the total volume was made up to 20 cc and was left over night. Ten cc of this solution was dialyzed against the same phosphate buffer solution. After the dialysis, the sample was centrifuged and the upper claer liquor was used for electrophoresis. The liquor was pH 6.98 and contained 0.38% of the concentration of the protein.

(3) Dil. NaOH solution-soluble Proteins

Twenty five thousandths Mol NaOH solution-extracted protein solution was adjusted to pH 4.2. The protein precipitated here was dialyzed against water and then centrifuged. About 4.5 g of the sediment of the protein was dissolved by phosphate buffer solution, of which the ionic strength was 0.35 and the total volume was made up to 18 cc. Ten cc of this solution was dialyzed againat phosphate buffer solution in the same manner as in (1) and (2), and was employed for the electrophoresis. This was pH 6.8 and contained 1.04% of the protein.

2 Electrophoretic examination

The electrophoresis was examined by the H. T. type Tiselius' apparatus. The size of the electrophoretic cell is $2\times15\times50$ mm (area of the section is 0.3 cm^2). The potential grade was 5.75 volt/cm for all samples. The temperature of the cell was maintained at 4°~6°C during the electrophoretic examination.

3 Experimental results

Results obtained are shown in Table 6–1 and Fig. 6–1.

Table 6–1. Protein components and the mobility of fractionated proteins

Fractionated proteins and its components		Mobility ($\times10^{-5}$cm^2/volt. sec.)	Remarks
Water soluble protein	Component I	−11.05	μ=0.15
	Comp. II	− 7.9	5.75 volt/cm
	Comp. III	− 7.05	pH 6.74
0.6 M. NaCl soln.-soluble protein	Comp. I	−24.3	μ=0.15
	Comp. II	−17.9	5.75 volt/cm
	Comp. III	−12.2	pH 6.98
0.025 M. NaOH soln.-soluble protein	Comp. I	−15.8	μ=0.35
	Comp. II	−14.3	5.75 volt/cm
	Comp. III	−13.6	pH 6.80
	Comp. IV	−11.5	

In Fig. 6–1, the descending boundary was used for the calculation of the mobility. As seen in Table 6–1 and Fig. 6–1, water soluble protein is composed of a mixture of three components, I, II and III which are ranked in order of the high magnitude of the mobility. Component I shows mobility of 11.05, II shows 9.7 and III shows 7.05×10^{-5} cm^2/volt sec. NaCl solution-soluble protein was also found to be composed of three components, I, II and III. Component I shows mobility of 24.3, II shows 17.9 and III shows 12.2×10^{-5} cm^2/volt. sec. The main components are regarded as II and III. Of those three, the mobility of component II is similar to that of component I of water soluble protein. Therefore, both those components are considered to be the same. Here, component I and II of which the mobilities are very high, were considered to be dissolved out from the extract by NaCl solution. For dil. NaOH solution-soluble protein, the electrophoresis was made using the buffer solution of ionic strength of 0.35. Therefore this fraction can not be compared in the values of mobility with the fractions of water soluble protein and NaCl solution-soluble protein of which the ionic strength of the buffer solution are 0.15. But as seen in Fig. 6–1, dil. NaOH solution-soluble protein is composed of four components, I, II, III and IV. Among them, the main components are regarded to be II and III of which the mobilities are 14.3 and 13.6 respectively.

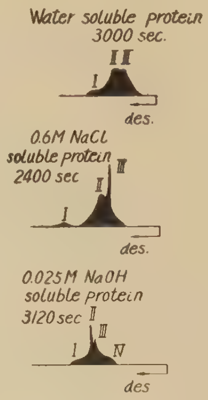


Fig. 6–1. Electrophoretic patterns of fractionated proteins

The results as above stated lead to the following conclusion. It is difficult to determine, from the present obtained results, which of components in water soluble protein corresponds to myogen, myoalbumin, globulin X, respectively, which are found ordinarily in the muscular protein, from the difference of the mobility. Because the mobility varies according to the preparation of the sample, pH and ionic strength of buffer solution, etc. As component III in NaCl solution-soluble protein is equal to component I in water soluble protein, it is considered to have been dissolved out abundantly. Component I in the same protein, has a mobility which is remarkably very large. In four components which were found in dil. NaOH solution-soluble protein, components II and III are regarded as the principal ones. To summarize, it was ascertained that water soluble protein has three components, NaCl solution-soluble protein has also three components, while dil. NaOH solution-soluble protein has four components.

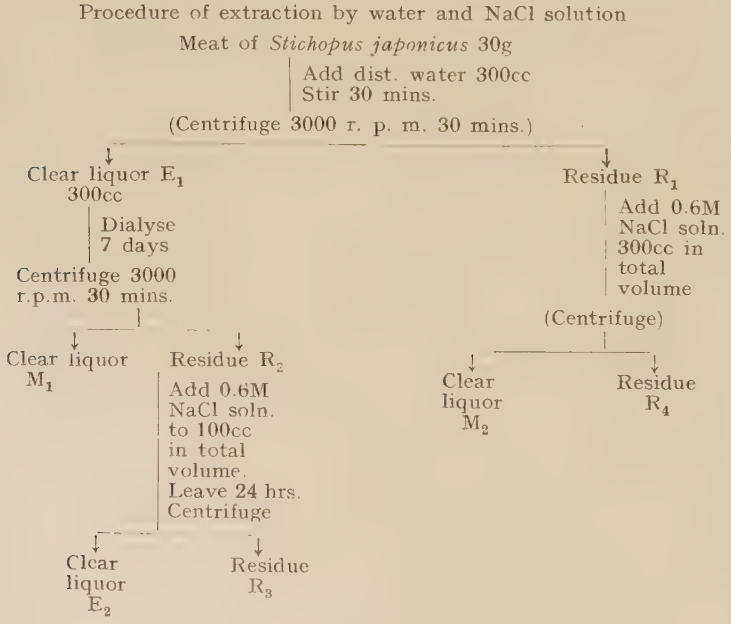
VII. PHENOMENON OF THE DISSOLUTION OF PROTEINS IN THE MEAT OF *STICHOPUS JAPONICUS*

It is of much interest that in properties of the proteins of the meat of *Stichopus japonicus*, the aqueous extract of the meat shows flow birefringence like squid meat. As known up until the present, in the muscular protein, the protein which shows flow birefringence is said to be myosins. The substance which shows flow birefringence in the squid meat is considered to be myosins[3]. The author has observed the phenomenon of flow birefringence and the solubility of the meat of *Stichopus japonicus* and has made estimation of the amount of nitrogen dissolved out, in order to know whether or not that appearance of flow birefringence is caused by myosins dissolved out in water from the meat. Myosin is not dissolved in water, but myosins may be considered to be dissolved out in water from the meat of *Stichopus japonicus* by the influence of the existence of meat extracts similarly to the case in squid meat.

1. The phenomenon of the dissolution of the meat by repeated extraction of 0.6 Mol NaCl solution

By a method similar to that used by Matsumoto[8][15] on the squid meat, the present author has extracted 30 g of the meat of *Stichopus japonicus* by 300 cc of water, and then extracted the residue repeated by 0.6 Mol NaCl solution. The amounts of the dissolved protein nitrogen were estimated as shown in Scheme 7–1.

Scheme 7–1.
Procedure of extraction by water and NaCl solution



As shown in Scheme 7–1, if the upper clear liquor centrifuged contains some sediment after the dialysis, there may be some dissolved globulin-like substances. If there is a part of "R_2" which is insoluble by 0.6 Mol NaCl solution in Scheme 7–1 and the part, "E_1", shows flow birefringence, myosins are considered to be dissolved in the part of "E_1". The results obtained are shown as Table 7–1.

Table 7–1. The amounts of dissolved protein nitrogen and the phenomena of flow birefringence of water and NaCl solution-extracted solutions

	Protein-N (mg) in 10cc extracted solution	$\frac{\text{Soluble protein-N}}{\text{Protein-N in 10g of Sample}} \times 100$ (%)	S.B. (Flow birefringence was abbreviated according to Migita *et al.*[18])
E_1	4.99mg	6.45	+
M_1	1.48	1.91	+
M_2	8.33	10.70	+
E_2	0.46	0.59	+

Note: The amount of protein nitrogen in 10 g of sample was 77.35 mg.

As seen in Table 7–1, aqueous extract "E_1" of the meat of *Stichopus japonicus*, the upper clear liquor "M_1" centrifuged after the dialysis of the residue of "E_1", 0.6 M NaCl solution-extracted solution "E_2" from the residue of "R_2", and the upper clear liquor "M_1" centrifuged after the extraction of the residue "M_2" by 0.6 Mol NaCl solution all showed flow birefringence. In the part "M_2", the amount of the dissolved protein nitrogen was the largest.

2. The phenomenon of the dissolution of the meat by repeated extractions with water

If the extracting time of the meat of *Stichopus japonicus* with water is short, the extract does not immediately show flow birefringence. The author has made repetition of aqueous extraction for each 10 minutes for the meat and observed the phenomenon of flow birefringence.

(1) Sample

Bodies of *Stichopus japonicus* which were caught in the sea near Hakodate early in June were divided into two parts, meat part and skin part as shown in Fig. 7–1 and were crushed.

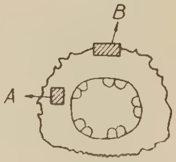


Fig. 7–1. Sampling parts (A: meat part, B: skin part)

(2) Experimental method

The crushed meat and skin parts were extracted repeatedly by twenty times volume of dist. water respectively, and centrifuged. The volume of the upper clear liquor was measured. Add 10 cc of 10 % trichloracetic acid to 20 cc of the liquor, and heat the mixture on a water bath. After heating, filter the mixture through a filter paper. The residue of the filtration was employed for the estimation of the amount of protein nitrogen. On the other hand,

the intensity of flow birefringence of the upper clear liquor, "S_1", "S_2", "S_3," was observed. For the detection of flow birefringence, an apparatus reported Okada and Tada[3] was employed. The extraction was repeated until no pattern of flow birefringence was observed. Sediments at the centrifugations were extracted by 1 Mol KCl solution (adjusted to pH 7.25 with 0.03 Mol $KHCO_3$) and 1 Mol LiCl solution, and the amount of protein nitrogen extracted was estimated as shown in Scheme 7–2.

Scheme 7–2.
Procedure of repeated extractions with water

Crushed meat of *Stichopus japonicus* 10 g
- Add dist. water 200 cc
- Stir 10 mins.
- Centrifuge (3000 r.p.m. 30 mins.)

Clear liquor (S_1)
- Total nitrogen
- Protein nitrogen
- S.B.

Precipitate (R_1)
- Add dist. water 200 cc
- Stir 10 mins.
- Centrifuge

Clear liquor (S_2)
- Total nitrogen
- Protein nitrogen
- S.B.

Precipitate (R_2)
- Treat as above

(3) Experimental result and discussion

Results obtained are shown in Table 7–2 and Table 7–3.

After the 3rd or 4th extraction of the meat of *Stichopus japonicus* with water, the patterns of the flow birefringence appeared in the meat and skin parts. That is to say, if the extraction was made for short time, the original substance causing the flow birefringence does not appear in the 2nd or 3rd extraction. The residue centrifuged from the aqueous extraction of meat or skin part shows a particular condition of sediment as shown in Fig. 7–2. According to Matsumoto[17], when the squid meat is extracted with 20 times volume of dist. water, the first extracted solution consists of three layers: A-layer which is an upper clear liquor, B-layer which is situated in the bottom of the centrifugal tube and contains white cloudy rod-like, thread-like or filament-like sediment which is supposed to be stroma, C-layer which is situated above B-layer and contains white semi-transparent gruel-like substance. After the second extraction, a small amount of sediment is formed in the upper part of C-layer, indicating liquidity, and if the tube is inclined, the liquid layer flows out in company with A-layer. Matsumoto calls it D-layer. In the case

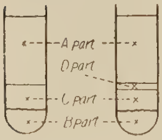


Fig. 7–2. appearance of centrifuged sediment of aqueous extracted solution.
A : Supernatant solution,
B : White opaque
C : White translucent, paste like,
D : Flow out by decantation

Table 7–2. Phenomenon of dissolution of the meat part of *Stichopus japonicus* during the repeated extractions with water

Extraction No.	Extracted soln. (cc)	Soluble protein-N in 10cc of extracted soln. (mg)	Per cent of soluble protein -N for protein -N in 10 g of sample	S.B.	pH	appearances of sediment
S_1	200	8.14	6.59	–	6.9	Kept in original form
S_2	198	.01	4.12	–	7.0	Ditto
S_3	194	5.01	4.12	–	6.9	Did not effuse by decantation.
S_4	179	7.12	7.56	–	7.3	Softening of meat, upper layer of sediment flowed out by decantation
S_5	175	13.58	10.99	++++	7.1	Further softening; D-layet piled on.
S_6	163	16.75	13.56	++++	7.2	Ditto, D-layer piled on in maximum
S_7	173	12.81	10.37	+++	7.0	No segment of meat
S_8	192	7.84	6.34	+++	7.1	All of the sediment flowed out by decantation
S_9	183	6.70	5.42	+++	7.0	Ditto
S_{10}	200	6.19	5.01	+	7.0	Ditto, D-layer decreased in volume
S_{11}	200	3.51	2.84	±	7.0	Ditto, decreased further
S_{12}	200	1.32	1.06	–	7.0	All of sedim. flow out by dec.
Sum		97.98	78.98			
1M KCl		2.39	1.93			
1M LiCl		1.26	1.02			
Residue		(29.04)	(23.51)			
Total sum		125.14	105.44			

Note: The amount of protein-N in 10g of sample was 123.5 mg.

of extracting of meat or skin parts of *Stichopus japonicus* with water, a similar phenomenon was observed by the present author as in Fig. 7–2. The observations are recorded in the last column of Table 7–2. In this case, the pattern of flow birefringence of the extracts appeared from 4th extract. The 6th extract of the meat part showed a distinct cross. The 9th extract of the skin part showed also the same. After 12th extraction, the pattern of flow birefringence of the extract of the meat part or skin part quite disappeared. According to Matsumoto[17], in extracting of squid meat with water, the pattern of flow birefringence appeared from the 2nd or 3rd extraction, the 4th extraction showed the most distinctly, and after the 6th extraction it disappeared.

Comparing the extraction of meat part or skin part of *Stichopus japonicus* with the meat of squid, the beginning of appearance of the pattern of flow birefringence of aqueous meat extract of the former was later and the continuance of its appearance through many extractions was longer than in the aqueous extract of squid. The

Table 7·3. Phenomenon of dissolution of the skin part of *Stichopus japonicus* during the repeated extractions with water

Extraction No.	Extracted solution (cc)	Soluble protein-N in 10cc of extracted soln. (mg)	Percent of Soluble protein-N for protein-N in 10 g of sample	S.B.	pH
S_1	198	9.48	2.16	–	8.4
S_2	190	0.04	0.04	–	8.5
S_3	184	2.14	2.74	–	8.9
S_4	168	7.86	9.70	±	9.0
S_5	184	4.66	5.98	+	8.8
S_6	174	0.04	0.04	++	8.6
S_7	185	2.08	2.66	++	8.6
S_8	184	2.12	2.72	++	8.1
S_9	180	3.04	3.90	+++	7.5
S_{10}	180	4.58	5.86	++	7.3
S_{11}	195	2.62	3.36	±	7.6
S_{12}	200	1.50	1.92	–	7.9
Sum		39.86	56.08		
1M KCl		3.60	4.62		
1M LiCl		2.64	3.39		
Residue		(32.56)	(41.79)		
Total sum		78.66	105.98		

Note: The amount of protein-N in 10g sample was 77.9 mg

reason for those facts is considered to be as follows: The tissue of the meat of *Stichopus japonicus* which consists mainly of a network of connective tissue is more difficult of destruction than the meat of squid. Total amount of the dissolved nitrogen of the extract of *Stichopus japonicus* through all rounds of the extraction is 79% for the meat part and 56% for the skin part. If the residue of the extracts of both parts was respectively extracted with 1 Mol KCl solution (adjusted to pH 7.25 by 0.03 Mol $KHCO_3$ solution) or 1 Mol LiCl solution, a colôrless transparent solution was obtained. That solution did not indicate a pattern of flow birefringence. The total amount of the dissolved nitrogen of the residue by 1 Mol KCl and 1 Mol LiCl was about 3% (meat part) and about 8% (skin part). The appearance of the residues of the meat part and skin part after each water extraction also corresponded closely to the condition of the C-layer of Matsumoto's experiment.

The substance which shows flow birefringence in the aqueous extract of squid is considered to be myosins[18] as above stated. Matsumoto[17] has considered the reason as follows: When 2nd aqueous extract, "S_1", of squid meat was dialyzed carefully for 1–2 days without the denaturation of the fraction of albumin, and was centrifuged, a part of proteins in 2nd extract, "S_2", precipitated. Next, when the sediment was suspended in water, the solution showed flow birefringence. The substance which shows flow birefringence seemed to be a protein belonging to the fraction of globulin. Myosins solution was obtained by extracting squid meat with 20 times volume of 1 Mol

KCl solution (adjusted to pH 7.25) and diluted to 0.05 Mol of the ion concentration and centrifuged; the sediment formed was suspended in water. The intensity of flow birefringence of the myosin solution thus obtained is the same as 2nd extract, "S_2", of squid meat. The interfering actions of KCl, LiCl, $MgCl_2$ and ethylalcohol for the appearance of flow birefringence of both solutions were the same. Here the two solutions of myosins and the 2nd extract, "S_2", were supposed to be the same. Then when the sediments of C-layer and the 2nd extract, "S_2", of the squid meat were dissolved in various concentrations of KCl solution, the smallest solubility was seen in 0.025 Mol KCl solution. Szent-György[19] has said that the protein which has the smallest solubility in 0.025 Mol KCl solution is myosin. For the reasons stated above, the substance which shows flow birefringence was determined to be myosins. In the meat of *Stichopus japonicus*, when 3rd and 4th aqueous extracts, "S_3" and "S_4" of the meat part and the skin part were carefully dialyzed, without the denaturation of the fraction of albumin, no sediment appeared. Next, when the crushed meat of *Stichopus japonicus* was extracted with 20 times volume of 1 Mol KCl solution (adjusted to pH 7.25) and then the extract was diluted to 0.05 Mol with water, and when the diluted solution was centrifuged, a small amount of sediment precipitated. It did not show flow birefringence. The 3rd and 4th extracts, "S_3" and "S_4" of *Stichopus japonicus* meat with water were also equal to the KCl extract in having little sediment and in the disappearance of the pattern of flow birefringence. Those facts prove that the substance which shows flow birefringence is not myosins, but may be other substance or chemical components in the meat or skin part of *Stichopus japonicus*. Those other substances or chemical components are supposed to be (A) chondroitin sufuric acid ester which combined with protein in the mucous substance in the meat, or (B) filamentous fragments of collagen fiber. Chondroitin sulfuric acid ester also shows flow birefringence[20]. The author will discuss in a later Article, what the substance may be which shows flow birefringence.

3. The phenomenon of the dissolution of the meat by repeated extractions with Weber's solution

Twenty g of the crushed meat part was repeatedly extracted with 200 cc of Weber's solution (0.6 Mol KCl, 0.04 Mol $NaHCO_3$, 0.01 Mol Na_2CO_3) and the phenomenon of the dissolution were observed. The result is shown in Table 7–4. In this case, as contrast, the squid meat was also treated in the same manner. The result is shown in Table 7–5.

As seen in the tables, in *Stichopus japonicus* meat, first extract with Weber's solution showed a faint cross in flow birefringence, 3rd extract showed the distinctest. After 6th extraction, the pattern of flow birefringence disappeared. On the other hand, in squid meat, the first extract with Weber's solution showed distinct flow

Table 7-4. Phenomenon of dissolution of the meat of *Stichopus japonicus* during repeated extractions with Weber's solution

Extraction No.	Soluble protein-N mg/200cc	$\frac{\text{Soluble protein-N}}{\text{Prot.-N in sample}} \times 100$ (%)	S.B.	Appearance of sediment
S_1	6.27	8.99	–	Upper layer of sediment flow out by dceantation
S_2	—	—	+ + +	Ditto
S_3	—	—	+ + + +	Upper and middle layers flowed out by decantation
S_4	—	—	+ + +	Ditto
S_5	—	—	+ + +	Suspended state, meat softend
S_6	—	—	–	No segment of the meat
S_7	—	—	–	All of the sediment lost by decatation
Residue	37.90	53.16		

Table 7-5. Phenomenon of dissolution of the meat of squid during the repeated extractions with Weber's solution

extraction No.	Soluble protein-N mg/200c.c	$\frac{\text{Soluble protein-N}}{\text{Prot.-N in sample}} \times 100$ (%)	S.B.
1	232.2	41.8	+ + + +
Residue	557.2	39.6	

birefringence as great as the 3rd extract of *Stichopus japonicus* meat. From those facts, it may be concluded that the substance which shows flow birefringence in *Stichopus japonicus* meat was extracted gradually ; on the other hand, the substance in squid meat was extracted from the first. The amount of the extracted residue of *Stichopus japonicus* meat is larger than that of squid meat. That is to say, the former meat is more difficult to dissolve than the latter meat. Those differences are remarkable.

4. The amount of the dissolved protein nitrogen from *Stichopus japonicus* meat by water with the various ratios of the amount of the extracting water to the amount of the meat part

Extractions of *Stichopus japonicus* meat were made with water in various ratios to the amount of meat. The extracts were centrifuged (3,000 r.p.m.). The amounts of the dissolved protein nitrogen were estimated with the results shown in Fig. 7–3 and Fig. 7–4.

As seen in Fig. 7–3 and Fig. 7–4, the amount of extracted protein nitrogen of *Stichopus japonicus* was small. The concentration of protein dissolved in the upper

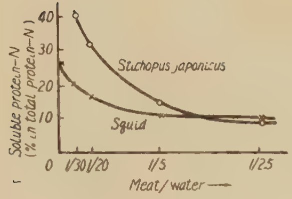


Fig. 7-3

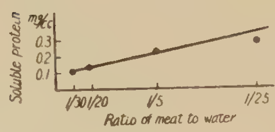


Fig. 7-4.

Fig. 7-3. The amount of protein nitrogen in supernatant solution when the ratio of meat to water was varied

Fig. 7-4. The amount of soluble protein nitrogen when the ratio of meat to water was varied

clear liquor increased with the increasing of the ratio of meat to water. Apart from the amount of the dissolved nitrogen, the condition of dissolving of meat of *Stichopus japonicus* is similar to that of squid meat which was studied by Matsumoto[17]. The curve showing the ratio of the amount of the dissolved protein nitrogen to the total amount of protein content is similar to that of squid meat. From those results, in the case of *Stichopus japonicus* meat also, the greater the amount of extracting water is, the greater the amount of the dissolved protein becomes. That is to say, the amount of protein soluble by water varies relatively by the extraction method and by the ratio of the amount of extracting water to the meat. The type of increase of the amount of dissolved protein of *Stichopus japonicus* meat and squid meat are different from the case of the fish meat, therefore, the constructions and properties of the former proteins are supposed to be comparatively different from the latter.

5. The increase and decrease of the intensity of flow birefringence when *Stichopus japonicus* meat was extracted with water of various ratios by volume to the meat and when the extracts were diluted in various concentration

At first, *Stichopus japonicus* meat was extracted with water of the various ratios by volume to the amount of the meat, viz., 1/20, 1/10, 1/5 and 1/2.5. The extracts were diluted with water of 2, 4, and 8 times volume. The relative intensity of flow birefringence in the diluted solutions was observed. If the intensity of flow birefringence was ordered as +++, ++, +, ±, ±, the intensity become gradually weak with increase in the degere of the dilution. Here, +: a distinct cross of pattern appears ; ± : bright dark areas are moving like a cluster of clouds, but do not form a distinct cross ; ± : the clouds are very faint. The increasing of numbers of + shows the increasing of the intensity of the flow birefringence. The amounts of protein nitrogen dissolved at the minimum concentration in which the patterns of the flow birefringence disappeared were mutually compared. Results are shown as Table 7-6 and Table 7-7.

Table 7-6. The increase and decrease of the intensity of flow birefringence when the meat part of *Stichopus japonicus* was extracted with water of various ratios by volume to the meat and when the extracts were diluted to various concentrations

Ratio of meat to water	S.B	Protein-N in 1cc of extracted soln. (mg)
1/20 ↓	+ +	0.064
1/40 ↓	+	0.035
1/80 ↓	±	0.021
1/160	±	0.019

Ratio of meat to water	S.B.	Protein-N in 1cc of xtracted soln. (mg)
1/10 ↓	+ + +	0.116
1/20 ↓	+ +	0.049
1/40 ↓	+	0.030
1/80	±	0.022

Ratio of meat to water	S.B	Protein-N in 1cc of extracted soln. (mg)
1/5 ↓	+ + + +	0.132
1/10 ↓	+ +	0.060
1/20 ↓	+	0.036
1/40 ↓	±	0.032
1/80	±	0.019

Ratio of meat to water	S.B.	Protein-N in 1cc of extracted soln. (mg)
1/2.5 ↓	+	0.109
1/5 ↓	±	0.059
1/10	±	0.032

Table 7-7. The increase and decrease of the intensity of flow birefringence when the skin part of *Stichopus japonicus* was extracted with water of various ratios by volume to the meat and the extracts were diluted to various concentrations

Ratio of meat to water	S.B.	Protein-N in 1cc of extracted soln. (mg)
1/5 ↓	+ + + +	0.166
1/10 ↓	+ +	0.091
1/20 ↓	+	0.043
1/40 ↓	±	0.035
1/80	±	0.022

Ratio of meat to water	S.B.	Protein-N in 1cc of extracted soln. (mg)
1/10 ↓	+ +	0.080
1/20 ↓	+	0.059
1/40 ↓	±	0.040
1/80	±	0.025

Ratio of meat to water	S.B	protein-N in 1cc of extracted soln. (mg)
1/20 ↓	±	0.026
1/40	±	0.023

Ratio of meat to water	S.B.	Protein-N in 1cc of extracted soln. (mg)
1/2.5 ↓	+	0.083
1/5 ↓	±	0.039
1/10	±	0.022

As seen in Table 7–6 and Table 7–7, in both the meat and the skin parts almost the same amount of dissolved protein nitrogen was estimated at the limit of the dilution

in which the flow birefringence shows ± or ±. But in the dilution, in which the flow birefringence shows + or over ++, the smaller the ratio of the amount of water to the meat is, the greater the amount of the dissolved protein nitrogen is. In the case of the extraction of the meat of *Stichopus japonicus* with water, to the suspended substance in C-layer, 3rd extract, "S_3", and 4th extract, "S_4", were added 1 Mol KCl solution in various volumes in order to make solution of 0.5, 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, 0.008, 0.006, 0.004, 0.002 and 0.001 Mol respectively. The solutions were left for 30 minutes. After standing, the amounts of protein nitrogen in the upper clear liquor of the centrifugation were estimated. The results obtained are shown in Figs. 7–5, 7–6.

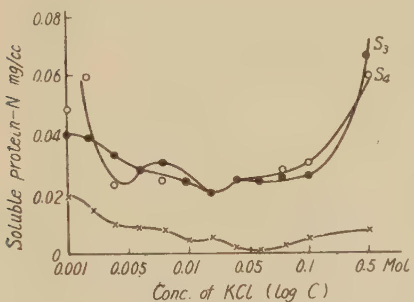


Fig. 7-5.

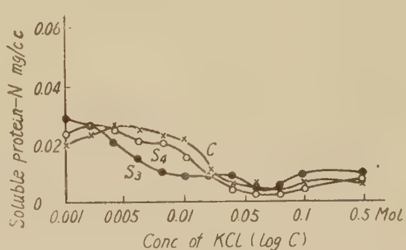


Fig. 7-6

Fig. 7-5. Comparative solubliity of the dissolved protein of the meat part of *Stichopus japonicus* in 3rd and 4th extracted solution with water and of the sediment of C-layer in various concentrations of KCl solution

Fig. 7-6. Comparative solubility of the dissolved protein of the skin part of *Stichopus japonicus* in 3rd and 4th extracted solution with water and of the sediment of C-layer in various concentrations of KCl solution

As seen in Figs. 7–5, 7–6, the solubility of protein in the meat part and skin part of *Stichopus japonicus* was the smallest in the concentrations of KCl solution between 0.02 and 0.06 Mol. The solubility of squid meat was the smallest in the concentrations of KCl solution between 0.02 and 0.04 Mol. There seemed to be a little difference between *Stichopus japonicus* and squid meat. From those solubilities, the phenomenon of flow birefringence observed in the meat of *Stichopus japonicus* is similar to that in the meat of squid, but is not seemed to be caused by myosins. Then what is the substance which shows flow birefringence in the meat of *Stichopus japonicus* ? This question will be answered later.

VIII. THE PHENOMENON OF FLOW BIREFRINGENCE OF THE WATER EXTRACT OF *STICHOPUS JAPONICUS* MEAT AND THE BEHAVIOUR OF THE ELECTROPHORESIS OF THE WATER EXTRACTED PROTEIN

In the previous Article, it was noted that when the meat of *Stichopus japonicus* was repeatedly extracted by water, flow birefringence was observed to appear beginning with the 3rd or the 4th extraction. It was stated that the substance which shows flow birefringence in the meat of *Stichopus japonicus* is not myosins. Even if the substance is not myosins, there may be some differences of the protein components between the no observed fraction of flow birefringence and the observed fraction of the aqueous extracts. For example, some protein may appear newly in the observed fraction of the aqueous extract. Here is reported an attempt to find the difference or the new appearance of protein component. For the sake of comparison, squid meat also has been employed.

(1) Preparation of samples

Add 300 cc of dist. water to 200 g of the fresh crushed meat of *Stichopus japonicus*, and shake the mixture for 30 minutes. Add 200 cc of dist. water to the mixture, and shake it. After the centrifugation of the mixture (4,000 r.p.m.), the flow birefringence of the upper clear liquor was observed. Then, add 300 cc of dist. water to the centrifuged residue from the treatment above described and shake it vigorously. Add 200 cc of dist. water to the mixture. After centrifugation, the flow birefringence of the upper clear liquor was observed. Thus the same treatment was repeated. When the flow birefringence appeared in the water extract, as a boundary of the appearance, the aqueous extracts of the fraction in which no flow birefringence was observed and the observed fraction were gathered separatedly until the flow birefringence disappeared. The aqueous extract of both fractions each was saturated with ammonium sulfate and the proteins were precipitated. After leaving over night, the precipitated proteins were separated by centrifugation. The precipitated protein were dialyzed against water for a week. The dialyzing porteins contained in the water were separated by centrifugation. The upper clear liquor in both fractions was negative for protein reactions by trichloracetic acid. Water soluble proteins thus obtained were used for the electrophoresis.

In the fraction in which no flow birefringence was observed, *S.B.* (−), the sediment of the total water soluble proteins was about 17 g as wet matter. Suspend the sediment in water, separate the solution by centrifugation. The upper clear centrifuged liquor showed no flow birefringence, and it was also negative for protein reaction by trichloracetic acid. This is due to the result that the water soluble proteins became partially insoluble. That is to say, the water soluble proteins are considered to be denaturated partially. Suspend about 4 g (as wet matter) of the

water soluble proteins in the phosphate buffer solution of which the ionic strength, μ, is 0.15. Make the total volume to 40 cc. Stand the suspended solution in a cool place over night. In this time, the most of the proteins were dissolved in solution. This protein solution was dialyzed against 500 cc of the same buffer solution having the ionic strength, μ, 0.15 (at 4°~10°C). After 48 hours' dialysis, the solution was centrifuged, and the upper liquor was employed for the electrophoretic examination.

In the fraction in which flow birefringence was observed, *S.B.*(+), about 86 g (as wet matter) of the sediment which was precipitated by ammonium sulfate was obtained. Suspend about 40 g of the sediment in the phosphate buffer solution, and make the total volume to 80 cc. After the solution had been left over night, 75 cc of it was dialyzed for 48 hours against 750 cc of the phosphate buffer solution of which the ionic strength, μ, was 0.15. After centrifugation of the dialyzed solution, the upper clear liquor showed flow birefringence, and it formed network-like sediment of fibrous substance. Next, the upper clear liquor was filtered with a filter paper, then the filtrate showed no flow birefringence. The filtrate was used for electrophoretic examination as the sample of *S.B.*(+) *A* (before the filtration, flow birefringence showed +, after the filtration −). Next, about 40 g of the sediment above as obtained was treated similarly up to the point of the dialysis against the phosphate buffer solution. This dialyzed solution has not been filtered. Of course, this solution showed flow birefringence. This was used for the electrophoretic examination as the sample *S.B.* (+) *B* (always flow birefringence showed +).

Squid meat was also treated in the same manner as *Stichopus japonicus* meat. That is to say, to 200 g of the crushed squid meat was added 300 cc of dist. water and the mixture was shaken. To the meat extract thus obtained was added 200 cc of water and the solution was centrifuged. When the meat extract which was obtained by the repeated extraction showed the flow birefringence, as a boundary of the appearance of flow birefringence each fraction was gathered respectively. Each farction was saturated with ammonium sulfate, and dialyzed for a week. Fraction in which no flow birefringence was observed was about 35 g (as wet matter) of the sediment. Ten g of the sediment in the fraction showing no flow birefringence was dissolved in the phosphate buffer solution of which the ionic strength, μ, is 0.15. This solution was made up to 40 cc in total volume. After the solution has been left over night, 20 cc of the solution was dialyzed against 500 cc of the same phosphate buffer solution, for 48 hours. After the dialysis, the solution was centrifuged. The upper clear centrifuged liquor was used for the electrophoretic examination. Aqueous extract of squid meat in the fraction showing flow birefringence was saturated with ammonium sulfate. This solution was dialyzed against water. After dialysis, it was centrifuged. In the centrifugal tube the material was seen to consist of three layers, *viz.*, a hard sediment was situated at the bottom of the tube, clear liquor

situated above the hard sediment and amorphously coagulated substance situated above the clear liquor. The middle clear liquor showed no flow birefringence, and showed (±) of protein reaction by trichloracetic acid solution. The bottom sediment and the amorphously coagulated substance above the clear liquor were used togather for electrophoresis. The sum of the two was 6.3 g (as wet matter). Forty g of the mixture was dissolved in the phosphate buffer solution of which the ionic strength, μ, was 0.15 and the total volume was made up to 150 cc. After having stood over night, 20 cc of the solution was dialyzed against 500 cc of the same buffer solution. After centrifugation, the upper clear centrifuged liquor showed no flow birefringence, and positive (+++) in the protein reaction by trichloracetic acid. The fact that the meat extract showed flow birefringence at first, but changed to show no flow birefringence after the treatment, is perhaps due to denaturation. For example, actomyosin which shows flow birefringence was dissociated to actin and L myosin, which show no flow birefringence[3]. Consequently, the partially denaturated proteins were examined in the electrophoresis. But if the degrees of denaturation were almost equal, the number of peaks and their mobilities are considered possibly relatively comparable in the same condition of electrophoresis.

(2) Electrophoretic examination

The electrophoresis was examined by the same H. T. Type Tiselius' apparatus as was used in the study reported in Article VI. The potential grade was 5.75 volt/cm for every sample. The phosphate buffer solution was prepared with 0.15 ionic strength and 7.38 pH value. The pH value of the sample for electrophoresis which was obtained after dialysis was about 7.0.

(3) Experimental result

The conditions of the appearance of flow birefringence in the repeated extractions of *Stichopus japonicus* and squid meat with water are shown in Table 8–1.

Table 8-1. The conditions of the appearance of flow birefringence in the repeated extractions of *Stichopus japonicus* and squid meat with water (Ratio of meat to water = 1 : 2.5)

Fractions & Extraction No.		S.B.	Fraction & Extraction No.		S.B.
Stichopus japonicus			Squid		
S.B. (−) fraction	1	−	S.B. (−) fraction	1	−
	2	−		2	−
	3	−		3	−
	4	−			
Stichopus japonicus			Squid		
S.B. (+) fraction	5	+	S.B. (+) fraction	4	+
	≀	≀		≀	≀
	14	+		11	+
	15	±		12	−
	16	−		13	−

As seen in the above table, in meat of *Stichopus japonicus*, the flow birefringence became to show from the 5th extraction, and it disappeared from the 16th extraction. In squid meat, the flow birefringence became to show from the 4th extraction, it disappeared from 12th extraction. In each sample most of the residue of both extracts which show no flow birefringence, consists of tough stroma. The aqueous extract of the fraction showing no flow birefringence was (±) for the protein reaction by trichloracetic acid. The results of electrophoretic examination for the various isolated proteins are shown in Table 8–2, Fig. 8–1 and Fig. 8–2.

Table 8 2. Protein components and the mobilities of aqueous extracted proteins of *Stichopus japonicus* and squid meat

Sample	Comp. I	Comp. II	Comp. III	Remarks
Stichopus japonicus S.B. (−)	−23.9	−18.7	−15.5	$\mu=0.15$ 5.75 volt/cm, pH 6.92 2700 sec.
Stichopus japonecus S.B. (+) A*		Comp. I −17.0	Comp. II −13.5	〃 〃 pH 6.84 3795 sec.
Stichopus japonicus S.B. (+) B	−22.0	—	Comp. II −11.0	〃 〃 pH 6.72 1260 sec.
Squid S.B. (−)	−7.5	−4.3		〃 〃 pH 6.60 3600 sec.
Squid S.B. (+)	−7.5	−2.9	−11.3	〃 〃 pH 6.71 3300 sec.

Note: Numerals in Table shows the mobility of each component, $\times 10^{-5}$cm^2/volt. sec.

* Fraction of S.B. (+) A is the fraction which show no flow birefringence.

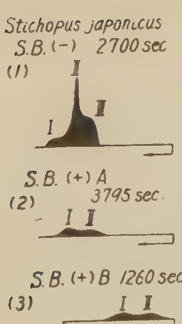


Fig. 8-1. Electrophoretic patterns of fractionated protein from *Stichopus japonicus* meat with repeated aqueous extraction (descending boundary)

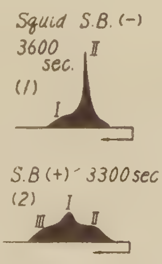


Fig. 8-2. Electrophoretic patterns of fractionated proteins from squid meat with repeated aqueous extraction (Ditto)

From Table 8–2, some very interesting facts may be noted. That is to say, as the boundary of the appearance of flow birefringence, although no component appeared in the meat extract of *Stichopus japonicus* with water, one component appeared newly in the aqueous extract of squid meat in which flow birefringence became to show. Fig. 8–1 (1) shows the descending boundary (2700 sec) of the fraction showing no flow birefringence in the meat extract of *Stichopus japonicus*, *S.B.*(—). From those patterns, the fraction is seen to contain three components, viz., component I shows 23.9 of mobility, II shows 18.7, III shows 15.5×10^{-5} cm^2/volt sec. Fig. 8–1 (2) shows the descending boundary of the fraction showing flow birefringence in the extract of *Stichopus japonicus* at first, and no flow birefringence after the filtration, *S.B.* (+)*A*. From those patterns, it is seen that this fraction contains two components, viz., component I shows 17.0 of mobility, II shows 13.5×10^{-5} cm^2/volt sec.

Comparing the former fraction, *S.B.*(—). with the latter fraction, *S.B.*(+)*A*, apart from a little difference of pH, the mobilities of components II and III of the former fraction are similar to those of components I and II of the latter fraction respectively, so they seem to correspond the same respectively. From those facts, it is considered that by the filtration of the aqueous extract of the latter fraction, the substance which corresponds to component I of the former fraction was removed. Fig. 8–1 (3) shows the descending boundary of the fraction showing flow birefringence in the extract of *Stichopus japonicus* without filtration. The descending limb of Fig. 8–1 (3) was obscure. This fraction contains two components, viz., component I which shows 22.0 of mobility, and II which shows 11.0×10^{-5} cm^2/volt sec. The obscure pattern is considered possibly to be due to the dilute concentration of the protein or to the suspension of some fibrous substance in the solution. The mobilities of the components of this fraction are different slightly from those of two fractions above stated. But if the difference is perhaps due to viscid properties of solution (owing to the suspension of fibrous matter), the descending limb of component I of this fraction is considered to be the sum of components I and II of the fraction showing no flow birefringence, *S.B.*(—). If one of components in the aqueous extract of the fraction showing flow birefringence at first, *S.B.* (+)*B*, was removed by the filtration, and if the removed component is considered to correspond to component I of the fraction showing no flow birefringence, *S.B.*(—), this component I is considered to have some connection with the suspended fibrous matter in the aqueous extract.

It was clarified from the electrophoretic pattern that there is a small amount of suspended fibrous matter in the aqueous extract of the fraction showing no flow birefringence, *S.B.*(—), but on account of the somewhat small amount of the suspended fibrous matter, flow birefringence was considered to have not shown. From those results as above obtained, in the aqueous extract of *Stichopus japonicus* when the extract shows flow birefringence changing from no flow birefringence, no new component appeared in the fraction showing flow birefringence, *S. B.*(+)*B*.

Next, will be treated the phenomenon of flow birefringence in the aqueous extract of squid. In the aqueous extract in which no flow birefringence is shown, *S.B.*(−), the water soluble proetin includes two components as shown Fig. 8–2 (1). The mobility of component I was 7.5 and that of II was 4.3×10^{-5} cm²/volt sec. On the other hand, in the aqu·ous extract fraction in which flow birefringence is shown, S.B.(+), the protein contains three components as shown in Fig. 8–2 (2). The mobility of component I was 7.5, II was 2.9 and III was 11.3×10^{-5} cm²/volt sec. Components I and II of the latter fraction, *S.B.*(+), are similar to components I and II of the former fraction, *S.B.*(−), respectively, judging from their mobilities, that is to say, they are considered to be the same respectively. From those facts, as the boundary of the appearance of flow birefringence, one component, III, appeared newly in the fraction of the aqueous extract of the meat of squid in which flow birefringence became to show, *S.B.*(+).

Summarizing results above described, in the aqueous extract of squid there was a difference of the new appearance of one component of protein having high mobility between the fractions which show no fl·w birefringence *S.B.*(−), and flow birefringence, *S.B.*(+). On the other hand, there was no distinct differance in the aqueous extract of *Stichopus japonicus* between the fractions which show no flow birefringence *S.B.*(−), and show flow birefringence, *S.B.*(+). From those results, the cause of the appearance of flow birefringence in the repeated extractions of squid meat with water is considered to originate from the dissolved protein, *viz.*, myosins[3)18)]. On the other hand, the cause in *Stichopus japonicus* meat is not the dissolved protein, but to be some other substance.

(4) General discussion on the appearance of flow birefringence in the aqueous extract of *Stichons japonicus meat*

As explained in regard to the phenomenon of the dissolution of meat protein of *Stichopus japonicus* in the previous Article, in the case of the repeated extractions of the meat with water, flow birefringence appears from the 4th extract ; this phenomenon continues to the 10th extract. After the disappearance of flow birefringence, the extract becomes comparatively transpsparent, and the residue of the extract suggests the conditions of insoluble stroma. Those phenomena are similar with those in squid meat[3)18)]. But some differences are observed between the two as follows.

(1) In the centrifuged liquor of the aqueous extract of *Stichopus japonicus* in which flow birefringence is observed, *S.B.*(+)*B*, white semi-transparent, minute fibrous matter is suspended. If the liquor is gently rotated in the air, the suspended matter flashes fluorescent light against the projected light. On the other hand, the centrifuged liquor of squid meat extract with water was slightly turbid, and even if it is rotated, there is no substance which flashes light against the projected light.

(2) If the centrifuged liquor in the aqueous extract of *Stichopus japonicus* in the

fraction of *S.B.*(+)*B*, is placed without rotation between two crossed Nicol prisms, a dark cross, the cross of isocline, is temporarily disturbed, but after the end of the rotation, a clear cross of isocline is again visible. In the case of the aqueous extract of squid, when the centrifuged liquor in which flow birefringence is the most dibtinct, the liquor shows the same phenomena without rotation, but the liquor shows the cross of isocline ordinarily after the rotation.

(3) If the aqueous extract of *Stichopus japonicus* in which flow birefringence has been observed was filtered through a filter paper, the filtrate does not show flow birefringence, *S.B.*(+)*A*. On the other hand, even if the aqueous extract of squid in which flow birefringence has been observed was filtered, the filtrate became transparent and showed more clear cross of isocline, *S.B.*(+). From results as above obtained, there is evidently some difference in the condition of the appearance of flow birefringence. As a factor for appearance of flow birefringence in the aqueous extract of *Stichopus japonicus*, the existence of the fibrous substance having polarizing action is necessary. This fibrous substance is easily removed by filtration. From the electrophoretic examination, there is no difference in the kind of dissolved proteins between fractions of *S.B.* (−), and *S.B.* (+) *B* in the aqueous extract of *Stichopus japonicus*. On the other hand, a component dissolved newly into the aqueous extract of squid meat which has become show flow birefringence. From the facts as above stated, the aqueous extract of *Stichopus japonicus* meat shows flow birefringence which is resulting without the presence of soluble proteins.

To summarize the progress to this point, the following subjects on the aqueous extract of *Stichopus japonicus* have been studied. Results obtained are briefly stated as follows : (1) When the queous extract of the meat which showed flow birefringence, *S.B.*(+), is filtered, the filtrate showed no flow birefringence, *S.B.*(+) *A*. (2) When the aqueous extract of the meat which showed flow birefringence, *S.B.*(+), is added with equal volume of 20% trichloracetic acid, a net-like sediment is formed in the solution. If the sediment is heated (2 minutes' boiling), it contracts and coagulates in one block. (3) When the aqueous extract of the meat which shows flow birefringence, *S.B.*(+), is heated (2 minutes' boiling), no heat-coagulated substance was formed, and fibrous matters remain, but the solution becomes to show no flow birefringence. (4) When the aqueous esxtract of the meat which shows flow birefringence, *S.B.*(+), is heated and then is added with 20% trichloracetic acid solution, the sediment formed is not a net-like substance, but it is a cloudy substance. (5) When to the aqueous extract of the meat which shows flow birefringence, *S.B.*(+), is added NaOH solution, fibrous matter is dissolved and the solution becomes transparent ; the solution shows no flow birefringence. When acids (HCl or H_2SO_4) are added to the solution as above obtained, white cloudy sediment is formed. (6) When the aqueous extract of the meat which shows flow birefringence, *S.B.*(+), is added Ehrlich's reagent or α-naphtol (Molish reaction reagent) which

are reagents for the identification of chondroitin sulfuric acid, the solution shows (±) in the reaction. The extract which has shown no flow birefringence, after the filtration, shows also (±). That is to say the reaction for chondroitin sulfuric acid is not remarkable. (7) When the aqueous extract of squid which shows flow birefringence is filtered, the solution shows it as before. (8) When the aqueous extract of squid meat which shows flow birefringence is added with 20% trichloracetic acid solution after heating (2 minutes' boiling) or without heating, white turbid cloudy sediment is formed in the solution. (After the solution is left standing over night, the upper liquor becomes transparent). When the solution is heated, the intensity of flow birefringence weakens (± or +). (9) The reaction of chondroitin sulfuric acid in the aqueous extract of squid meat which shows flow birefringence is not remarkable, (±).

From the experimental results as above described, the results numbered (1) and (2) suggest that the fibrous substances in aqueous extract of *Stichopus japonicus* meat which shows flow birefringence constitute a network with their thread-like fiber. Results numbered (3) means that the fibrous substances are not coagulated by heating, but their thread-like fiber is finely cut and suspended irregularly, consequently the flow birefringence is not seen. Results (4) and (5) indicate that the cloudy sediment is formed by the addition of trichloracetic acid after heating. This is perhaps because the fibrous substances are easily dissolved by alkali, losing their threadlike construction, and become show no flow birefringence ; then cloudy sediment will be formed in the solution by the addition of acids. Results (6) – (8) show that due to the absence of the proteins which causes positive reaction of chondroitin sulfuric acid and shows flow birefringence, the substance which is responsible for the occurrence of flow birefringence in *Stichopus japonicus* meat is neither myosins nor a combined protein substances with chondoroitin sulfuric acid, but it is considered rather reasonably to be suspended matters which are fibrous substances having thread-like shape. Of course, even if the suspended matter is changed to cloudy sediment by the addition of trichloracetic acid after heating, it cannot be said positively that the heated substance has not necessarily a thread-like construction. But at least, from the fact that in only case of forming a network-like sediment by the addition of trichloracetic acid, the presence of the sediment is necessary for the appearance of flow birefringence, it can be said positively* that the network-like sediment is formed from the thread-like substance.

* Gelatine-gels consist of filamentous protein molecules having network construction, and are unoriented isotropic fibers. If gelatine gels are subjected to mechanical tension so that their protein threads are regularly oriented, and they become birefringent. (Haurowitz:Chemistry and Biology of Proteins, 1950, p. 184).

That the collagen consists essentially of thread-like particles is proved by the birefringence shown by the fibers in polarized light. The positive sign of birefringence indicates that the anisotropic particles are submicroscopic rodlets, arranged parallel to the longitudinal axis of the fiber. (F.O. Schmitt: Advances in Protein Chemistry, 1, 25 (1944)).

In the case of squid meat, from the facts that even if the aqueous extract of the meat which is birefringent is filtered through a filter paper, the extract shows flow birefringence, the reaction of chondroitin sulfuric acid is (±), and the new appearance of one component in the birefringent extract ocucrs, the substance which shows flow birefringence is considered to be some protein having thread-like construction (*e.g.* myosins)[18].

Then, what is the substance which shows flow birefringence in the aqueous extract of *Stichopus japonicus* meat? The following answer to this question is proposed: From the facts that the suspended matter in the extract is not coagulated by heating, and that it is semi-macroscopic in size (dia. about 6 μ, several cm long), the substance is composed of filamentous fragments which were cut from the collagen fiber of the connective tissue by the repeated extractions with water, and is suspended dispersedly in the solution. As seen in those experimental results, the meat tissue (connective tissue) of *Stichopus japonicus* is easily broken down by the repeated extractions with water. This fact is considered to be reasonable from the following experimental results on the thermal stability of the flow birefringence of the aqueous extract. When the meat is extracted repeatedly with water of ten times its volume, and when each part of the 4th aqueous extract which is clearly observed flow birefringence is heated at 40°C or 70°C for 10 minutes, both the extracts show positive flow birefringence after the heating. Thus the substance which shows flow birefringence is considered to be stable against heating. Next, when the meat is extracted repeatedly by Weber's solution of ten times volume similarly to the above, the first extract shows flow birefringence like the results described in Article VII. If this extract is filtered through a filter paper, the filtrate shows (±) in flow birefringence. If this filtrate is heated, it shows (±) in flow birefringence upon heating for 10 minutes at 35°C, but (±) at 45°C, (−) at between 55°C and 75°C. In the case of the extraction by Weber's solution, the longer the extracting time, the more intense the degree of flow birefringence. The 3rd and 4th extracts show (+++) in flow birefringence, the 5th and 6th extracts show (++), the 8th extract shows (±), after 9th extract show (−). If the 3rd or 4th extracts which show the strongest flow birefringence are heated for 10 minutes at 40°C, the extracts show (++) in flow birefringence, but show (−) at 70°C. In the case of the extraction of the meat by Weber's solution, myosins which are considered to exist in the network of collagen fibers of the connective tissue of the meat of *Stichopus japonicus* are considered to dissolve out, so the extracted solution shows the more distinct flow birefringence. The fact that when Weber's solution-soluble extract is heated for 10 minutes above 45°C which corresponds to heat coagulating temperatures of myosins, the extracts shows no flow birefringence indicates that the substance which is responsible for showing flow birefringence is considered to be due principally to the existence of dissolved myosins.

On the contrary, the fact that the aqueous extract of the meat of *Stichopus japonicus* is stable against heating as 70°C for 10 minutes and shows flow birefringence after the heating, supports the conclusion that the substance which shows flow birefringence is not soluble proteins such as myosins, but is the suspended matter of collagen fibers in the solution. If the collagen fiber becomes mechanically loose as a result of extraction and the small pieces of collagen fiber suspended in the solution show flow birefringence, the aqueous extract of the residue which was extracted by Weber's solution and from which the flow birefringence disappeared, should show flow birefringence. Accordingly, each residue, namely the one which was left after the first extraction of *Stichopus japonicus* meat with Weber's solution, and the one which was left after the 9th repeated extractions of the separated *Stichopus japonicus* meat with Weber's solution of ten times volume and came to show no flow birefringence, — each of these residues was washed with water until the disappearing of KCl reaction. Then the residues were respectively again repeatedly extracted with water of 10 times volume. The intensity of flow birefringence of each extract was measured and then each extract was heated at 40°C or 70°C for 10 minutes and the intensity of flow birefringence after the heating was observed. Results obtained are shown in Table 8–3.

Table 8–3. The phenomenon of flow birefringence and its stability against heating of extracts from the formerly extracted residue with Weber's solution again extracted repeatedly with water

	Residue of 1st extract with Weber's soln.			Residue of 9th extract with Weber's soln.		
	original extract	after heating at 40°C, 10min	after heating at 70°C, 10min	original extract	after heating at 40°C, 10min	after heating at 70°C, 10min
S_1	+ +	+	−	+ + +	+ +	+ −
S_2	+ + + +	+	+ −	+ + +	+	+ −
S_4	+ + + + +	+ +	+ −	S_3: +		
S_5	+ + +	+ +	+ −			
S_6	+	−	−			
S_7	−					

As seen in Table 8–3, when the first residue and the 9th residue which were extracted with Weber's solution were extracted repeatedly with water, both water extracts of the residue show distinct flow birefringence (+ + + +). Stabilities are shown after heating at 70°C, and here again the residues show flow birefringence in the same degree as in the extract with Weber's solution only. The fact that the aqueous extract of the first residue after extraction with Weber's solution is thermostable to heating at 40°C and that it showed flow birefringence is considered evidence that myosins dissolved by the extraction with Weber's solution still remain after water washing, that they are suspended in the following water extract and that they show flow birefringence. But when the aqueous extract is heated at 70°C, the extract

changed to show no flow birefringence as a result of heat denaturation of the suspended myosins. The facts that in the course of repeated water extractions after the extraction with Weber's solution, the intensity of the flow birefringence of the aqueous extract increases and that the stability against heating increases with the increased number of water extractions support with still stronger evidence the conclusion as above stated. The thermostability of the aqueous extract of squid meat in respect to flow birefringence has also been observed by Okada[3] to be stronger than that of fish meat. He has observed that when the aqueous extract of squid meat which showed flow birefringence is heated at 40°C or 70°C for 5 minutes, the extract shows flow birefringence as before. Further, he has said that there are two substances which show flow birefringence in the aqueous extract after heating at either below or above 40°C. The substance which shows flow birefringcnce after the heating below 40°C is considered to be myosins according to the results obtained in the electorphoretic experiment reported above (Article VIII) and according to the results obtained from Migita and Matsumoto[18]. However, the substance which shows flow brefringence after heating at 40°C to 70°C, is supposed to be suspended matter of pieces of broken collagen fiber as well as the meat of *Stichopus japonicus*. This supposition is based upon the fact that connective tissue composed of collagen fibers in the squid meat was observed in the microscopic observations by Migita and Tanaka[21] or Niiyama[22]. Another basis of the supposition is the existence of a larger amount of collagen in squid meat than in fish meat (Article X).

Finally, the phenomenon of the breaking down of the meat tissue is also observed in the natural break down of the bodies of *Stichopus japonicus*[2]. If the bodies of *Stichopus japonicus* are left in the room, especially on the straw, or if they are frequently handled, the bodies are deformed, and the amount of mucous substance increases. The mucous substance is dissolved by the addition of alkali, and the dissloved matter is again coagulated by the addition of acid. If this coagulate is spread as a thin layer on a slide glass and is dried in the air, the dried filamentous matter is easily skinned from the glass. This filamentous matter is not dispersed and is not dissolved in water. On the other hand, if the suspended matter in the aqueous extract of *Stichopus japonicus* meat which shows flow birefringence, is filtered through a filter paper and if the residue is spread as a thin layer on the slide glass and dried, the filamentous matter was again easily skinned off. This filamentous matter was dissolved by the addition of alkali and coagulated by acid. This is also not dispersed and it is not dissolved in water. Both the filamentous matters are fibrous tissue substances having the same diameter of 2–6 μ and they have network construction at a certain part of the tissue under the microscope. From those results, the two substances are considered to be the same. In this Article, the meat of *Stichopus japonicus* was ascertained to consist mainly of collagen fibers of connective tissue.

IX. HEAT CONTRACTION OF THE MEAT OF *STICHOPUS JAPONICUS*

When the meat of *Stichopus japonicus* is heated in water, the meat shrinks more remarkable than fish meat. This phenomenon is perhaps due to the difference of the kind of the tissue constructing the body meat. That is to say, the tissue of *Stichopus japonicus* meat is collagen tissue, but the fish meat is muscular tissue. The author has tried to ascertain from the viewpoint of the heat contraction of the meat whether the tissue of *Stichopus japonicus* is collagen tissue.

1. In the case of heating the meat which includes radial canals

(1) Sample

The body of *Stichopus japonicus* was split at the ventral side, and eviscerated. The skin together with the upper layer of the meat was removed. Next, eight meat blocks of trapezoid shape as shown in Fig. 9–1 were made from a body. In this case, radial canal which runs lengthwise was attached under the meat block. Sixteen pieces of those meat blocks were prepared.

(2) Experimental method

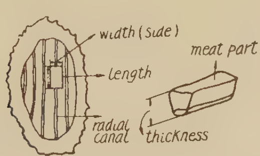


Fig. 9-1　Sample used

Before immersing of the meat block, the length, width and thickness (height) were measured. The blocks were immersed in water heated at intervals of 5°C between 20°C and 95°C. The immersion period was 10 minutes after the moment at which the definite desired temperature was obtained. After the heating, the block was taken up and the moisture on the surface was absorbed gently with a piece of filter paper. The length, width and thickness of the heated block were measured. The ratio (%) of the weights before and after the heating is considered to indicate the heat contractility. The smaller the value of contractility is, the larger is the degree of shrinkage of the meat block.

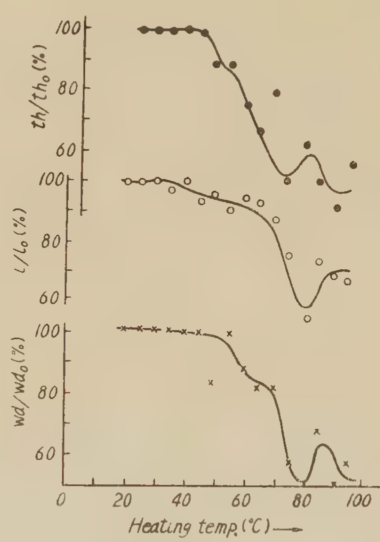


Fig. 9–2.　Relation between the contractility of *Stichopus japonicus* with radial canal and the heating temperature

(3) Experimental results

Results obtained are shown in Fig. 9–2.

The greatest contractility was observed in the thickness of the meat block. The width and length in order follow the thickness. The contractility of thickness of the block showed no change at temperatures between 20°C and 45°C. At higher temperatures the contractility becomes rapidly larger. Above 70°C the contractility showed about 50%. The contractility of the width of meat block showed no change between 20°C and 50°C, but above 55°C shrinkage began and at near 80°C the contractility showed about 47%. The contractility of the length began to be noticeable from near 40°C, and gradual shrinkage continued to 70°C. Above 70°, rapid shrinkage in length was observed. The contractility of the length was about 55% at 80°C. That contractility is slightly larger than that of the thickness and width. As above stated, the meat begin to shrink rapidly when immersed in water at temperatures between 45°C and 55°C and showed about 50% of contractility at 70°~80°C. Takahashi[23] has observed that the fish skin which is composed mainly from collagen begin to shrink from 37° ~55°C, and that shark skin (*Prionace glauca*)[24] showed about 50% of contractility at 53°C. The great contractility of the meat of *Stichopus japonicus* is similar with that of shark skin which is composed mainly of collagen. In fact, the meat of *Stichopus japonicus* is histologically connective tissue; that is to say, the meat is ascertained to be collagen fiber. Beside collgen, the heat coagulabilitics of myogen and myosin which may be present in the network of collagen fiber should be considered. Those subjects will be discussed later. The beginning temperature (70°C) of rapid contraction of the length of *Stichopus japonicus* meat is higher than that of width and thickness. This is very interesting. The cause is considered to be the inclusion of radial canal which runs lengthwise to the meat block in this experiment. The contraction of the radial canal exerts much influence upon the contraction of length of the meat block by heating.

2. In the case of heating the meat without radial canal

(1) Sample and method

Two bodies of *Stichopus japonicus* were split at ventral side, and eviscerated. The skin with the upper layer of the meat was removed. In this case radial canals were carefully removed. Eight pieces of meat blocks without radial canal were prepared. This blocks were subjected to the same treatment as above described. For comparison and contrast, the removed radial canal was measured for contractility by the same method.

(2) Experimental results

Results obtained are shown in Fig. 9–3.

As seen in Fig. 9–3, the contraction of the length, width and thickness began at about 30°C. The contractions of the width and thickness continued rapidly until 65°C, at which temperature the heat contractions showed 55~60%. The contraction

of the length of the meat block without radial canal showed slowly until 75°C when it was about 75%. The contractions of the length, width and thickness of the meat block were almost equal. The characteristic slow contraction seen in the length of the meat block with radial canal was not seen in the contraction of length of the meat block without radial canal. The heat contraction of the length of the radial canal itself began at 30°C and continued slowly until 90°C. This presents evidence that the characteristic contraction of the length of the meat with radial canal may be considered to be due to the attachment of radial canal.

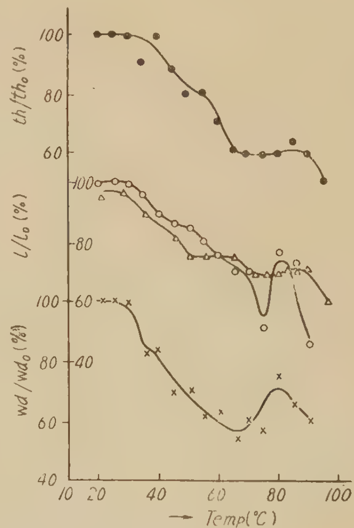


Fig. 9-3. Relation between the contractility of *Stichopus japonicus* meat without radial canal and the heating temperature

From results as above described, the heat contraction of the meat block began at 30°C, the greatest contractility was shown as 50~60% between 70° and 80°C. The fact that the heat contractions of the length, width and thickness of the meat of *Stichopus japonicus* were almost equal as learned in Experiment 2, is evidence of the network construction of collagen fiber, of which the tissue fibers are arranged equally length-ways and side-ways.

X. THE AMOUNT OF COLLAGEN IN THE MEAT OF *STICHOPUS JAPONICUS*

From the viewpoint of histology, the meat of *Stichopus japonicus* was ascertained to be mainly connective tissue. Such tissue was observed to form a network construction. From the experiments on solubility, flow birefringence and heat contraction, the connective tissue is considered to be collagen fiber. Here, the author has estimated the amount of collagen in the meat of *Stichopus japonicus* and fish, and observed the difference between them.

(1) Sample

Bodies of *Stichopus japonicus* were split on the ventral side, and samples were prepared from (A) the upper skin layer, (B) dorsal meat (between the skin part and the coelum), and (C) ventral meat (between the ventral skin part and the coelum) as shown in Fig. 10–1. The samples of meat were curshed for the estimation of the amount of collagen. As contrast, five sorts of fish meat were used: Atka mackerel, (*Pleurogrammus azonas* JORDAN et METZ) Soi-fish (a kind of gray rock cod, *Sebastichthys trivitatus* HIRGENDORF), flat fish (*Limanda herzensteinii* JORDAN et

SNYDER), carp (*Cyprinus carpio*) and squid (*Ommastrephes sloani pacificus*). Gelatinized samples prepared by Mitchell's method[25], were employed for the estimation of the amount of gelatine-nitrogen by Kashiwada and Kakimoto's method[26]. Put each 10 g of the crushed samples into Erlenmeyer's flasks containing 100 cc of dist. water respectively. Plug the flask with cotton. Heat the sample in the autoclave at 110°C for 90 minutes. Add 20% trichloracetic acid solution to the heated sample in order to precipitate protein. After filtration, the filtrate was neutralized with NaOH solution. Add 10% lead acetate to the neutralized filtrate in order to precipitate the comparative larger molecules of nitrogen compounds, *e.g.* pepton and polypeptides. After the filtration, add 5 cc of 2N H_2SO_4 to remove the excess of lead acetate, and neutralize the solution. Add Folin-Wu reagent (10 cc of 10 % sodium tungstate and 10 cc of 2N H_2SO_4 solution) to the neutralized solution in order to precipitate only gelatine. After the washing of the precipitant with N/5 H_2SO_4 solution, the amount of gelatine-nitrogen was estimated by Kjeldahl's method. Results obtained are manifested by the amount of gelatine-nitrogen. Besides gelatine-nitrogen the amount of water content and total nitrogen were estimated by the usual method.

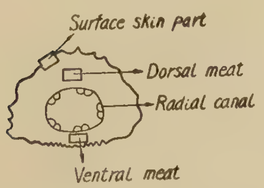


Fig. 10-1. Sampling parts

(2) Experimental results

Results obtained are shown in Table 10–1.

As seen in Table 10–1, the amount of gelatine-nitrogen in the meat of *Stichopus japonicus* is 0.014~0.034% of the original matter, though there is some difference according to the body parts from which samples were taken. For comparision of meat of *Stichopus japonicus* with other fish meat excepting squid meat, the minimum amount (0.013% for carp), the maximum amount (0.046% for Atka mackerel) were estimated for the original matters. *Stichopus japonicus* meat has a larger amount of

Table 10-1. The amount of collagen in the meat of *Stichopus japonicus* and that of other fish meat (calculated from gelatine-nitrogen).

Samples \ Items	Water content in raw meat (%)	Total nitrogen in raw meat (%)	Gelatine-N in raw meat (%)	Gelatine-N per dried matter (%)	Gelatine-N in Total nitrogen (%)
Stichopus japonicus upper skin layer (A)	88.92	0.88	0.034	0.307	3.86
Dorsal meat	88.25	1.09	0.027	0.230	2.48
Ventral meat (C)	87.00	0.97	0.014	0.108	1.44
Atka mackerel	78.65	2.73	0.046	0.215	1.69
Soi-fish	79.73	2.84	0.028	0.138	0.99
Flat fish	82.66	2.54	0.021	0.121	0.83
Carp	79.21	2.50	0.012	0.063	0.52
Squid	75.14	3.00	0.105	0.422	3.50

water content (87–89%), and a smaller amount of total nitrogen (about 1% of the original, 7~9% of the dried matter) as its characteristics. On the other hand, fish meat shows 75~85% water content, and 2.5~3% as the total amount of nitrogen (12~17% of the dried matter). If the ratio (%) of the amount of the gelatine-nitrogen to the dried matter or to the total amount of nitrogen is calculated, in *Stichopus japonicus* meat it comes to 0.1~0.3% of the dried matter, 1.4~3.9% of the amount of the total nitrogen ; in fish meats 0.06% (carp)~0.2% (Atka mackerel) of the dried matter, 0.5% (carp)~1.7% (Atka mackerel) of the total amount of nitrogen. That is to say, the ratios in *Stichopus japonicus* meat are larger than those of other fish meat. Especially, the value of the ratio of gelatine-nitrogen to the total amount of nitrogen is two or three times that of other fish meat. In squid meat, the amount of gelatine-nitrogen is 0.4% of the dried matter, and 3.5% of the amount of total nitrogen. Those percentages are remarkably similar to those of *Stichopus japonicus*, that is to say those percentages are larger than those of fish meat. In the meat of *Stichopus japonicus*, the amount of collagen is different by position of the sample taking. In (A) part (the upper skin layer) the amount of collagen is the largest ; (B) part (dorsal meat) and (C) part (ventral meat)in order follow after the (A) part. The fact that the (A) part has the largest amount of collagen, is considered to have some connection with the surface skin, derma.

The fact that the meat of *Stichopus japonicus* has larger amount of gelatine-nitrogen than other fish meat (except squid) gives an experimental basis to the cosideration that the meat of *Stichopus japonicus* consists of connective tissue in the form of collagen fiber having network construction. Therefore, there seem to be dissolved certain kinds of proteins (*e.g.* myogen, myosin, etc.) in the network of collagen fiber. As observed in the previous experiment on the heat contraction the facts that the meat of *Stichopus japonicus* begins to shrink rapidly at 40°~50°C and that the contractility is very large, about 50%, show that the meat consists of connective tissue, a collagen fiber, in contrast with Takahashi's results[23]) that fish skin, which consists mainly of collagen fiber, begins to shrink at 35°~55°C and the contractility is large. Upon the contraction of collagen fiber in the tissue of *Stichopus japonicus*, protein and a part of the water in the network of collagen fiber seemed to escape. In the meat of squid there exists a comparatively large amount of gelatine-nitrogen as in *Stichopus japonicus*. This may be due to the presence of connective tissue which is made up mainly of collagen and which is oriented in a definite direction in the meat, as observed in the histological experiment[22]). From the above observations, it is clear that the meat of *Stichopus japonicus* is remarkably different from other fish meat in both chemical composition and tissue construction.

XI. DEHYDRATION OF THE MEAT OF *STICHOPUS JAPONICUS*

When fish meat or fish meat protein are cooked in water or salt solution, the meat or protein coagulates and is dehydrated. Simidu[27] has studied the dehydrating curve of fish muscle. He observed that the dehydrating curve is divided into two parts, one part corresponds with the coagulating point of myogen, the other part corresponds with that of myosin. The coagulating point in the dehydrating curve has been observed to move according to decline in the freshness of fish meat or the environmental temperature of living fish. Takagi[28] has also observed the heat coagulation of the water soluble proteins. He has stated that there are two kinds of protein having different heat coagulating points and that the coagulating point moves according to the environmental temperature of the fish habitat or migrating properties of fish, has also observed the influences of salts upon the heat coagulation. However, Simidu[29], upon reexamining the dehydration of loach fish meat (*Cobitis taenia* Linné) of various degrees of freshness, observed that there is only one heat coagulable protein in the fish meat. The hydrating power of this protein increases by heating or salting of fish meat and is lost by heating of high temperature or the preservation of long time. That is to say, he has corrected the previous experimental results. The present author has observed the dehydrating curve of fresh meat of *Stichopus japonicus* and of the soluble proteins during the heat coagulation.

1. Relation between dehydration and heating temperature

(1) Sample

Fresh meat of *Stichopus japonicus* which was caught in the sea near Hakodate was used for the experiment. Blocks of the meat, 1~2 cm^3 (4~6 g), were prepared. Each block was heated in 50 cc dist. water or 50 cc 1 Mol NaCl solution at definite temperature intervals from 25° to 90°C for 10 minutes. The weight of each block before and after heating was measured and the ratio of the dehydration was calculated by the following equation, $\frac{W_0-W}{W_0} \times 100$ (%), where, W_0 is the weight of the meat block before heating, W is the weight of the meat block after heating.

(2) Experimental results

Results obtained are shown in Fig. 11–1.

Curve I of Fig. 11–1 show the ratio of dehydration of the meat block heated in dist. water, while curve V of Fig. 11–1 show the ratio of dehydration of the block heated in 1 Mol NaCl solution. In Fig. 11–1, the dehydrating curve of Atka mackerel (*Pleurogrammus azonas*) meat heated in dist. water (curve II) obtained by Fujii[7] and those of living loach (*Cobitis taenia*) (curve III) and salmon (*Oncorhynchus keta*) (curve IV) heated in dist. water by Simidu[27][28] are drawn for the sake of comparison with the meat of *Stichopus japonicus*.

As seen in Fig. 11–1, the meat of loach and Atka mackerel show hydration rather

than dehydration below the coagulating point of the protein, and then show dehydration at temperatures above that point. However, when the block of *Stichopus japonicus* meat is heated in dist. water at various temperatures, the dehydration goes slowly until 30°~45°C, and then rapidly between 45°C and 90°C. When the meat of *Stichopus japonicus* is heated in 1 Mol NaCl solution, as seen in curve V of Fig. 11–1, the dehydration goes slowly until 40°C, and rapidly above 40°C. This fact agrees with the result obtained in case of heating in dist. water. The facts that curve V is situated above curve I, and that the ratio of dehydration in NaCl solution is smaller than that in dist. water are considered to agree with the absorption phenomena[30] as was observed in the salting of the meat especially in dilute solution of NaCl.

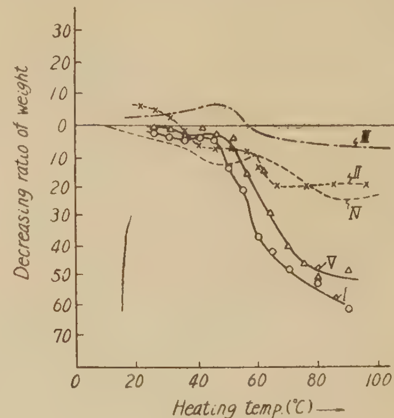


Fig. 11–1. Dehydrating curves of *Stichopus japonicus* and other fish meat

In the former experiment of Simidu[27], it was observed that the dehydrating curve of fresh salmon meat (curve IV in Fig. 11–1) increases gradually from 30°C to near 62.5°C, and rapidly from 65°C to 73°C. Simidu has explained this stage of different coagulating temperatures. He considered that the former protein which coagulates from 30°C to 62.5°C corresponds to Fürth's myosin[32], and the latter protein which coagulates from 65°~73°C corresponds to myogen. On the other hand, the dehydrating curve of Atka mackerel meat (curve II in Fig. 11–1) shows slow increase from 35°C to 45°C, and rapid from 55° to 65°C. That is to say, there seemed to be two kinds of proteins. One of them corresponds to myosin and the other corresponds to myogen. The coagulating temperatures of salmon protein are higher than those of Atka mackerel. This is perhaps due to the difference of the amount of protein content or to other properties according to the kinds of fish. However, in any case, the protein obtained in the salmon is almost similar to that of Atka mackerel. In the case of the meat of *Stichopus japonicus*, only one protein, which coagulates at 30°~45°C in dist. water or NaCl solution, was observed. No protein which coagulates at higher temperatures was observed. That is to say, the presence of the protein which coagulates at higher temperatures is a matter of question. This fact indicates a remarkable difference between *Stichopus japonicus* meat and fish meat of which the coagulable proteins are supposed to be myosin and myogen. Here, in the dehydrating curve of the meat of *Stichopus joponicus*, if a peak indicating myosin, of which the coagulating temperature is 30°~45°C (or soluble myogen fibrin of which the coagulating temperature is about 30°C) is observed, the peak of another water soluble protein, myogen, should appear

on the dehydrating curve. However, no such corresponding peak is observable. This is difficult to explain. Also as seen in Fig. 11–1, the meat of *Stichopus japonicus* is remarkably dehydrated to higher degree than the fish meat for which data are available. That is to say, the meat of *Stichopus japonicus* is intrinsically different from fish meat in the coagulating phenomenon brought about by heating. When the meat of *Stichopus japonicus* is heated, the phenomenon of contraction of collagen fibers which compose the connective tissue of the main part of the meat, is the most striking reaction. Then, the fact that the meat of *Stichopus japonicus* is dehydrated rapidly above 45°C, and is dehydrated in larger amount than the fish meat indicates principally the contraction of collagen fibers composing the connective tissue of the meat. This consideration may be supported by Takahashi's result[23] that the heat contracting temperature of collagen fiber is 35°–55°C. Therefore, the coagulation phenomenon of the soluble and coagulable proteins, *e.g.* myosin and myogen which seem to exist in the network of collagen fiber may not appear distinctly on the dehydring curve. Here, fractionated protein solutions, *viz.*, water soluble protein and 0.6 Mol NaCl solution–soluble protein were employed for the calculation of heat coagulating curve.

2. Relation between the heating temperature and the heat coagulation of water soluble protein, and 0.6 Mol NaCl solution-soluble protein

In the extraction of water soluble protein from the meat of *Stichopus japonicus*, there were found to be slight differences in the amount of dissolved protein nitrogen obtained at the various extracting times of 0.5 hour to 24 hours. In the extraction of NaCl solution-soluble protein, there were some slight differences in the amounts of protein nitrogen dissolved among the concentrations above 0.6 Mol of NaCl solution (Article III). In view of those obtained results, the meat of *Stichopus japonicus* was extracted by means of dist. water or by 0.6 Mol NaCl solution of three times volume for 24 hours. The amount of water soluble protein nitrogen obtained was 1.83 mg% by water extraction. In the water soluble protein nitrogen, the heat coagulable nitrogen was only 2.7% (this is 0.05 mg% of the original meat). The amount of 0.6 Mol NaCl solution-soluble protein nitrogen was 2.65 mg% in case of extraction by 0.6 Mol NaCl solution. In the 0.6 Mol NaCl solution-soluble protein, the heat coagulable nitrogen was 3.8% (this is 0.1 mg% of the original). Next, add 300 cc of dist. water or 0.6 Mol NaCl solution to 100 g samples of the meat of *Stichopus japonicus* respectively, and stand them for 24 hours. After that period, the mixtures were filtered through 1 cm of pulp layer. The filtrates contain water soluble protein or NaCl solution-soluble protein respectively. The total amount of nitrogen in 10 cc of the filtrates of both proteins was estimated. Fifteen cc portions of the filtrate of both solutions were heated at various temperatures and filtered. The total amount of nitrogen was estimated. The difference between the total amount of nitrogen in 10 cc of the original

solution of protein and 10 cc of the heated filtrate corresponds to amount of coagulable protein nitrogen. Either the amount of the coagulable nitrogen at comparatively high temperatures in the water soluble protein was not quantitatively estimated, or no precipitant was observed. The ratios of the coagulable protein nitrogen to the total amount of nitrogen in NaCl solution-soluble protein are shown as curve I in Fig. 11–2.

The heat coagulating curve of 0.6 Mol NaCl solution-soluble protein shows a sharp incline with two observable peaks at about 40° and 60°C. Curves II and III respectively are the heat coagulating curves of the water soluble proteins of squid meat and flat fish meat obtained by Takagi[28]. As may be seen from the heat coagulating curve of squid meat protein, there are two proteins. The coagulation of the one protein begins at about 40°C and ends at 55°C: that of the other begins at about 65°C and ends at 80°C. In the heat coagulating curve of flat fish meat protein, there are also two proteins. The coagulation of one begins at about 35°C and ends at 57.5°C; that of the other begins at about 65°C and ends at 80°C. In this case, a part of the myosin is supposed to dissolve out from the muscle tissue under the presence of a slight amount of salts in the muscle. From this fact, there are perhaps two stages in the heat coagulating curve owing to the existence of myosin and myogen which have different coagulating temperatures. Curves IV and V are the heat coagulating curves of the water soluble protein or 0.6 Mol NaCl solution-soluble protein of Atka mackerel meat respectively which were obtained by Fujii[7]. Curve VI showing the heat coagulating curve of the water soluble protein of Atka mackerel meat presents a smooth curve in the range between 30° and 70°C differing from the material from *Stichopus japonicus* meat of the author or squid meat and flat fish meat of Takagi[28]. In that curve there is supposed to be only one peak corresponding to myogen at about 70°C. Curve V showing the heat coagulating curve of 0.6 Mol NaCl solution-soluble protein of Atka mackerel meat shows two peaks. One of them is supposed to correspond to the existence of the first group of proteins of which the heat coagulating point is 30°–50°C, whilst the other peak is supposed to correspond to the existence of the second group of proteins which coagulate at 65°–75°C. As above stated, in the water soluble protein of Atka mackerel there was only one kind of protein. This finding is considered to agree with Simidu's result that there is primarily only one kind of protein in fish meat (*cf.* the results of the heat coagulating of loach shown as

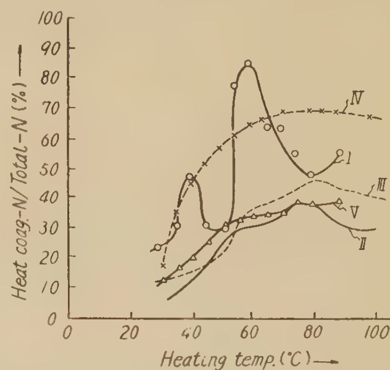


Fig. 11–2. Change of the amount of heat coagulable nitrogen when the extracted solution with 0.6 Mol NaCl was heated to certain intervals of temperature. (Total-N in 10 cc of the solution of *Stichopus japonicus* was 0.741 mg)

curve III of Fig. 11–1). But, in this case, myosin was supposed difficult to dissolve out from the meat on account of the very diluted salt solution in the meat. Therefore, in this experiment it seems reasonable that the two kinds of protein which are found in 0.6 Mol NaCl solution soluble protein in the meat of *Stichopus japonicus* correspond first to myosin of which the heat coagulating temperature is about 40°C and second to myogen of which the heat coagulating temperature is 65°~70°C. Those two kinds of proteins seemingly exist in the network of collagen fibers.

3. Variation of the dehydrating curve with the falling of freshness of the meat of *Stichopus japonicus*

The author has observed that the dehydrating curve of fresh meat of *Stichopus japonicus* shows rapid increasing above 45°C, and he has attributed the cause to the existence of the main protein constituent of collagen fiber which increases the contracting coagulation.

Examination was made as to just how the dehydrating curve of the meat of *Stichopus japonicus* changes with the falling of freshness.

(1) Experimental method

Fresh meat of *Stichopus japonicus* was left at room temperature (about 20°C) for 24 hours and 48 hours and three samples of the meat having various degrees of the freshness were prepared. The heat coagulation of the meat samples in dist. water was examined by the previously described experimental method.

(2) Experimental results

Results obtained are shown in Fig. 11–3.

As seen from curve I of Fig. 11–3, the dehydration curve is gradual until 40°C similarly to curve I of Fig. 11–1 and then if indicates rapid dehydration between 45°C and 85°C. In this curve, the phenomenon of heat contraction of collagen fiber which coagulates at 45°C is supposed to exert great influence. As seen in curves II and III, in the dehydration curves corresponding to the dehydration of the meat of various degrees of freshness from leaving for 24 hours and 48 hours, rapid dehydration was accelerated above about 45°C, that is to say, the existence of protein which coagulates above 45°C was not clearly observed. Above 45°C, greater dehydration was observed with the falling of the freshness of the meat, that is to say, with the softening of the tissue of the meat. Below 45°C, there was no great change in the dehydration. As shown by

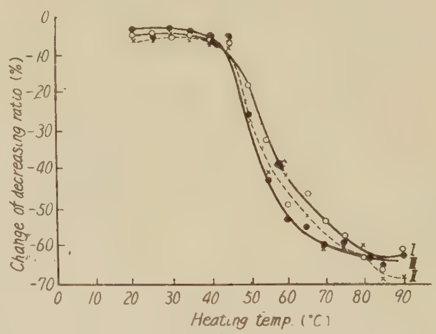


Fig. 11–3. Dehydrating curves of *Stichopus japonicus* meat in various degrees of freshness

the results above obtained, when the meat of *Stichopus japonicus* is heated in dist. water or 0.6 Mol NaCl solution at various temperatures, above 45°C a more rapid dehydration is produced owing mainly to the heat contraction of collagen fiber constituting the connective tissue of the meat. From results of experiments on the phenomenon of heat coagulation of 0.6 Mol NaCl solution-soluble protein of the meat of *Stichopus japonicus*, there seem to exist proteins corresponding to myosin and myogen. That is to say, myosin and myogen proteins are supposed to exist in the network of collagen fiber constituting of the meat.

XII. ACID COAGULATION OF THE MEAT OF *STICHOPUS JAPONICUS*

When the meat of *Stichopus japonicus* is immersed in vinegar, the meat becomes hardened. This is due to the coagulation of the meat protein. The author has observed the phenomenon of the acid coagulation of the meat of *Stichopus japonicus* and examined its properties.

(1) Sample

Blocks of the meat of *Stichopus japonicus* (3~5 g) were employed as samples.

(2) Experimental methods

Blocks of the meat were immersed in various kinds of acid solutions, (CH_3COOH, H_2SO_4 and HCl) having various concentrations (0.01~2.0 N). The weights of the block before and after the immersing in acid solutions were measured. The amount of nitrogen dissolved by the immersion was also estimated. Meat blocks of 3~5 g were accurately weighed. Each weighed block was immersed in 50 cc of the acid solutions having various concentrations at room temperature for 24 hours. In each case the upper liquor in the bottle in which the block was immersed, was estimated for the amount of dissolved nitrogen. The acid solution on the surface of the block taken up from the immersing solution was absorbed by filter paper. The wiped block was accurately weighed. Then, the ratio ($S=W/W_0$) of the weight (W) of the block after the immersion to the weight (W_0) of the block before the immersion was calculated.

(3) Experimental results

Results obtained are shown in Fig. 12–1.

As seen in Fig. 12–1, when the meat of *Stichopus japonicus* is immersed in acid solution, the meat coagulates. In the case of dilute acid solutions, the values of "S" ($=W/W_0$) decrease rapidly with the increasing of the concentration. In this case, the values of "S" show minimum between 0.01 and 0.02 N of H_2SO_4 and CH_3COOH solutions, respectively, and between 0.04~0.05 N of HCl solution.

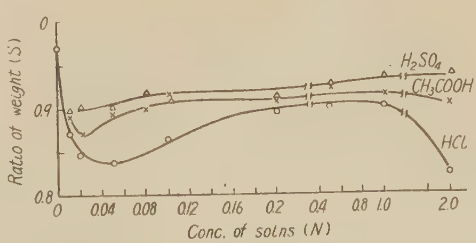


Fig. 12–1. Changes of the ratio of weight of *Stichopus japonicus* meat immersed in acid solutions having various concentrations

In the case of acid solutions of greater concentration, the values of "*S*" increase gradually but the degree of increasing becomes slow above 0.1 N of H_2SO_4 and CH_3COOH solutions respectively. In HCl solution, the value of "*S*" increases from the concentration of 0.05 N, and shows the maximum between 0.5 and 0.6 N, but above that range, the value decreases rapidly with the increasing of the concentration. Comparing three kinds of acid, the values of "*S*" become smaller in the order of H_2SO_4, CH_3COOH and HCl through various concentrations. That is to say, the degree of acid coagulation is the strongest in HCl solution and with CH_3COOH and H_2SO_4 solutions following in order. The results of estimations of the amounts of dissolved nitrogen from the coagulated meat immersed in various kinds of acid solution of 0.1~1.0 N, and the ratios of those amounts to the amount of total nitrogen in the raw meat are shown in Fig. 12–2. As seen in Fig. 12–2, in the case of H_2SO_4 solution, the amount of the dissolved nitrogen increases rapidly above 0.6 N, and shows constant below 0.6 N. In the case of HCl solution, the amount shows almost constant until near 0.4 N, and increases rapidly between 0.4 and 0.6 N, and then shows decrease at higher concentration. In the case of CH_3COOH solution, the amount shows almost constant through all the concentrations, and in the range of the concentrations used in this experiment, no rapid increasing was observed. According to Takahashi[32], the relation between the values of "*S*" and the amounts of the dissolved nitrogen is parallel, except CH_3COOH solution. In other words, the increasing of the amount of the dissolved nitrogen means the increasing of the values of "*S*". Those facts have been observed by Noguchi.[9] In the present author's results the findings that the value of "*S*" shows maximum in the concentration between 0.5 and 0.6 N and the amount of the dissolved nitrogen shows the maximum at 0.6 N in the case of the immersion in HCl solution, are in agreement with those of Takahashi and Noguchi. In the case of the immersion in H_2SO_4 solution, the value of "*S*" was observed to increase in the course of increasing of the amount of the dissolved nitrogen as seen in Fig. 12–1. In the case of the immersion in CH_3COOH solution, the amount of the dissolved nitrogen shows almost constant, as well as the constant increasing of the value of '*S*". According to Takahashi[32], with the increasing of the concentration of CH_3COOH solution (the decreasing of the value of pH), the amount of dissolved nitrogen increases in the case of H_2SO_4 and HCl solutions, while the amounts of the dissolved nitrogen decrease contrarily in solutions above

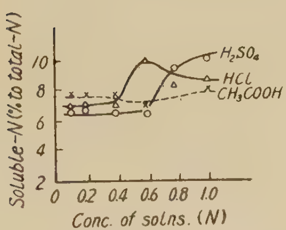


Fig. 12–2. Relation between the amounts of nitrogen dissolved from *Stichopus japonicus* meat and the concentrations of various kinds of acid solutions

Note: The total amount of nitrogen in raw meat is 0.83 % in case of the immersion in H_2SO_4 and HCl solutions, and that is 0.85% in the immersion of CH_3COOH solution.

certain concentrations (below pH 2.0).

In the present author's experiment, if still higher concentrations of the solutions were employed, the increasing of the dissolved nitrogen may be observed. The facts that the amount of the dissolved nitrogen shows constant until 0.4 N of acid solutions as seen in Fig. 12–2, and that the value of "*S*" shows the minimum until 0.4 N of acid solutions (except H_2SO_4 solution) as seen in Fig. 12–1, indicate that the meat of *Stichopus japonicus* is coagulated at first, and then the coagulated meat is considered to swell with the increasing of the concentration of acid solution. As observed in Article I, the meat of *Stichopus japonicus* consists of connective tissue which is composed of a network of collagen fibers, in which various kinds of protein are considered to dissolve or to combine with water. The existence of collagen fibers may play an important role in the acid coagulation of the meat. Likewise the phenomena of heat contraction and the dehydration of the meat of *Stichopus japonicus* may be affected mainly due to the existence of the collagen fiber as shown in Articles X and XI. According to Takahashi[32], in the experiment on acid coagulation of shark skin, the maximum swelling of the skin of which the principal component is collagen fiber has been observed at near pH 2.0. Generally speaking, protein has two peaks of swelling in acidic side (pH 2~4) and alkali side (pH 10~12) owing to the presence of diamino acid and dicarboxylic acid in the molecule[33]. The most diluted HCl solution employed in the present experiment corresponds to about pH 2. The fact that, in the case of the immersion in HCl solution, the value of "*S*" and the amount of the dissolved nitrogen show then maxima at 0.5~0.6 N, indicates that the maximum swelling of the meat of *Stichopus japonicus* is below pH 2.0. The 0.1 N CH_3COOH solution corresponds to about pH 3.8 and 0.8 N CH_3COOH solution has about pH 2.0. The result that the values of "*S*" are almost equal between 0.1 and 0.8 N of CH_3COOH solution shows that there is no distinct maximum swelling at near pH 2.0. The same conclusion is reached in the case of H_2SO_4 solution. The author has therefore examined in greater detail the relation between the swelling of the meat of *Stichopus japonicus* and the values of pH in the following Article.

XIII. THE SWELLING OF THE MEAT OF *STICHOPUS JAPONICUS*

In as much as the swelling of protein is considered to be one of the phenomena of lyotropic hydration, the author has examined the phenomenon of the swelling of the meat of *Stichopus japonicus* immersed in various salt solutions, and of the relation between the degree of swelling and the pH value of the immersed solution. Below are described the examination of the swelling of the meat of *Stichopus japonicus* immersed in various salt solutions of various concentrations, and the difference of the swelling owing to the difference of the freshness of the meat. There is also a discussion of the swelling as influenced by charged ions of salts.

1. The swelling of the meat immersed in salt solutions of various concentrations

Blocks of meat (2 cm³) were taken from the bodies of *Stichopus japonicus* which were caught in the sea near Hakodate and were accurately weighed. Immerse each block in 50 cc of the following various salt solutions at room temperature for 24 hours. After taking up, moisture on the surface of the blocks was wiped off with a filter paper, and the weight of the block after the immersion was weighed. The ratio of the weight (W) of the block meat after immersion to the weight (W_0) of the block meat before immersion was calculated as the degree of swelling (S) of the block of meat ($S=W/W_0$). Salt solutions employed were NaCl, KCl, KI, KNO_3, K_2SO_4, KCNS, $NaNO_3$, Na_2CO_3, NH_4Cl, $CaCl_2$, $MgCl_2$, $Na_3PO_4 \cdot 12H_2O$, CH_3COONa and $C_3H_4OH\ (COONa)_3$ solutions of 0.1, 0.2, 0.4, 0.8, 1.0 and 2.0 Mol and $Na_2B_4O_7 \cdot 10H_2O$ solution of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 Mol. As contrast, dist. water was also employed.

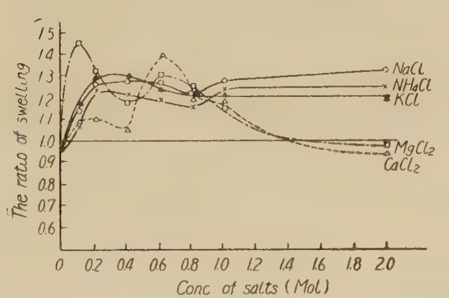


Fig. 13-1. The degree of swelling of *Stichopus japonicus* meat in various concentrations of solutions containing chlorides

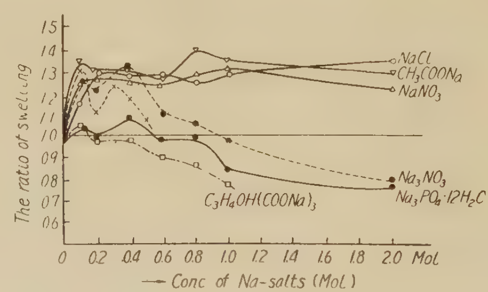


Fig. 13-2. The degree of swelling of *Stichopus japonicus* meat in various concentrations of solutions containing sodium salts

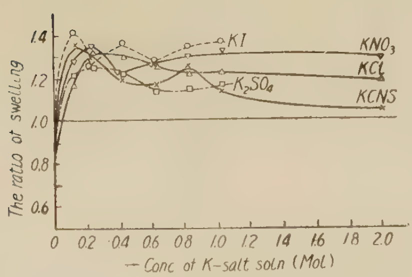


Fig. 13-3. The degree of swelling of *Stichopus japonicus* meat in various concentrations of solutions containing potassium salts

(2) Experimental results

The relation between the concentration of chlorides and the degree of swelling is shown in Fig. 13–1. Similarly, for sodium salts, Fig. 13–2, for potassium salts, Fig. 13–3.

As seen in Fig. 13–1, concerning the influence of varying cations upon Cl^-, in monovalent salts such as NaCl, NH_4Cl, KCl, the difference of degree of swelling in the salt solutions is proportionate to the difference of the concentration of the solutions. The degree of the swelling of the block meat shows the maximum at near 0.2 Mol of monovalent salt solutions, and it shows almost constant until 2 Mol of the solution. In NH_4Cl solution the second peak in degree of swelling is observed at near 1.0 Mol. On the other hand,

the degrees of swelling of the block meat immersed in the bivalent salt solution such as $MgCl_2$ and $CaCl_2$ are different remarkably from the swelling in the monovalent salt solutions. There are two peaks in the bivalent salt solutions: The first peak of maximum value of the degree of swelling is shown between 0.1 and 0.2 Mol and the second peak is shown near 0.6 Mol. The value of the degree of the swelling in the bivalent salt solutions is smaller than that in the monovalent salt solutions at the same concentration. That is to say, as seen in Figs. 13–2 and 13–3, there is some difference between the monovalent cation and the bivalent cation. In the case of monovalent cation solutions two peaks are shown at 0.2 Mol and 1.0 Mol. (In the KCl and NaCl solutions, the peak at 1.0 Mol can not distinctly observed.) In the case of the bivalent cation solutions, two peaks are distinctly observed at such lower concentrations as 0.1 and 0.6 Mol; further, the strong influence of hydration by the electrochemical adsorption of dissociated ion is observed. To generalize on the influence of anion in sodium salts from Fig. 13–2, monovalent anions such as NaCl, CH_3COONa and $NaNO_3$ make the first group and bivalent anions such as Na_3PO_4, $C_3H_4OH(COONa)_3$ make the third group. The degree of the swelling caused by monovalent anions is the greatest through their various concentrations, while bivalent anions and trivalent anions follow in order. In the case of swelling of the meat blocks immersed in the monovalent anion solutions, there are three peaks at near 0.1, 0.2 Mol and between 0.3 and 0.4 Mol similar to what is shown in Fig. 13–1. Considering the influence of the anion in potassium salt from Fig. 13–3, as the author has employ only a salt with bivalent anion, K_2SO_4, it was not possible to observe the difference between the influences by bivalent and monovalent anions. In the case of monovalent anions, KCl solution has a peak at near 0.2 Mol, KNO_3 solution has two peaks at 0.2 Mol and 1.0 Mol, KCNS solution has two peaks at near 0.2 Mol and 0.8 Mol, KI solution has three peaks at near 0.1 Mol, 0.4 Mol and between 1.0 and 2.0 Mol. On the other hand, K_2SO_4 solution has a peak at near 0.2 Mol in this experiment, but the presence of another peak is supposed in a higher concentration of this salt. As seen in Figs. 13–1, 13–2 and 13–3, when the meat of *Stichopus japonicus* is immersed in dist. water, the degree of the swelling (S) shows 0.97. That is to say, there was no remarkable absorbing phenomenon in *Stichopus japonicus* meat differing from the case of fish meat[27]. This fact was observed in the experiment of dehydration of the meat of *Stichopus japonicus* described above in Article XII. Thus the absorbing power of the meat of *Stichopus japonicus* is shown to be smaller than that of fish meat.

(3) Discussion

The degree of the swelling of meat of *Stichopus japonicus* is different with differences in the concentrations of the various salt solutions into which it is immersed, as above stated. The results obtained from experimental observations on this point are summarized as follows: 1) In the range of 0.2–2.0 Mol of the concentration of salt solutions, the greater the valency of the salt, the samller the degree of the swelling

of the meat is. 2) In the salt solutions of monovalent salt, there are two peaks in the degree of the swelling of the meat at near 0.2 Mol and at 0.8~1.0 Mol. 3) In the salt solutions of bivalent and trivalent salts, the peaks of the degree of the swelling of the meat slip to the lower concentration, and have two points at near 0.1~0.2 Mol and near 0.4~0.6 Mol. 4) In dist. water, the meat does not absorb water. 5) The influences of the cations and anions of the salt solutions upon the degree of the swelling of the meat show no difference according to their valency.

In contrast to the fact that Atka mackerel meat has one peak in the degree of swelling upon being immersed in salt solutions of various concentrations[7], it is noteworthy that the meat of *Stichopus japonicus* has two peaks in the degree of swelling in certain concentrations of salt solutions. In contrast to the fact that Atka mackerel meat shows the osmotic dehydration phenomenon at near 1.0~2.0 Mol of monovalent salt solutions, and absorbs water in dist. water showing 1.25 of the value of "*S*", that is to say, that the phenomenon of absorption was distinctly observed, it is characteristic that the meat of *Stichopus japonicus* absorbs dist. water in very small amount—less than fish meat does. Also, if the meat is caused to swell because of the existence of salts, the swollen meat becomes antagonistic to the osmotic dehydrating effect accompanied with the increasing of the concentration of salt solution. When as principal properties of coexisting ions, beside osmotic phenomenon in company with the increasing of the concentration of salt solutions (in the range of higher concentration), valency effect of ion (direct effect) and lyotropic effect (indirect effect) at middle or lower concentration are considered[34], the change from the direct effect to the indirect effect is made gradually, and at the middle concentrations of the salt solutions, both sorts of effects are shown in duplicate. In the case of the meat of *Stichopus japonicus* the observed fact that the meat has two peaks in the degree of swelling is considered to be due to the double effect.

2. The influence of the hydrogen ion concentration on the degree of the swelling of the meat of *Stichopus japonicus*

The swelling of the meat is a phenomenon due to the increasing of the volume by the pressure of water which penetrates into the mycel of the protein molecule by osmosis and by the affinity of water with the molecule, and then hydrates completely with the protein molecule as colloid. In this case, if salts exist in water, the salt linkage of the protein molecule is broken, and anion and cation associate with positively charged ammonium groups NH_3^+ and negatively charged groups COO^- respectively. Then the anion and cation accelerate the hydration of protein molecule in which they take a leading part. In this case, each ion either positively or negatively charged orients to the dissociated radicals of protein in the much lower concentration of the salt solution. With a slight increasing of the concentration the dissociated ions are adsorbed by surface chemical action by the protein molecule, and the hydration is

accelerated. That the hydration of colloid is influenced remarkably by an electrolyte is said to be due to the indirect increasing of the hydration of colloid by drawing up water in the hydration of electrolyte ion which was adsorbed by colloid. From another viewpoint, the swelling of a macromolecular substance such as protein is said to be intrinstically the first step in dissolution, and as it is regarded to be a kind of phenomenon of solvation,[35] as above stated, the charged ions are considered to be the center of the hydration. It is the hydrogen ion concentration that the number of charged radicals mostly influences. The influences of pH on the swelling of the fish meat has been previously studied by Tarr,[36] and recently by Okada and Tada.[10] They have observed that the degree of the swelling of fish meat is influenced by the hydrogen ion concentration. The present author has studied the influence of pH on the swelling of the meat of *Stichopus japonicus* in solutions of various pH values.

(1) Experimental method

Meat blocks (1.5 cm³) were taken from the fresh bodies of *Stichopus japonicus* as samples. pH of the immersing solution was adjusted by varying the mixing proportion of N/25 HCl and N/25 NaOH or N/25 H_2SO_4 and N/25 NaOH covering the range of pH 1 to 13. Each meat block (about 2~4 g) accurately weighed was put in a flask containing 50 cc of the solution of various pH and the flask was stood at room temperature (12°~16°C) for 24 hours. After that standing the meat cube only was taken up, and the moisture on the surface of the block was wiped off carefully with a filter paper. After the wiping, the weight (W) of the immersed block was measured, and the value of the degree of the swelling (S) of the block, *i.e.* the ratio of the weight, "W", to the weight (W_0) of the block before the immersion was calculated by the equation as above described. ($S=W/W_0$) Then pH of each solution after the immersion was estimated. KCl was added to each solution of various pH to make the solution of 0.6 Mol KCl. The swelling of the meat block in those solutions was examined.

(2) Experimental result

Results obtained are shown in Fig. 13–4. Curves I and II in Fig. 13–4 show the swelling of the meat of *Stichopus japonicus* in various pH solutions which were adjusted by N/25 H_2SO_4 and N/25 NaOH or N/25 HCl and N/25 NaOH solutions. Curves III and IV in Fig. 13–4 show the swelling curves of the meat in various pH solutions which were adjusted by N/25 H_2SO_4 and N/25 NaOH or N/25 HCl and N/25 NaOH solutions and to which was added KCl to make the concentration of 0.6 Mol KCl.

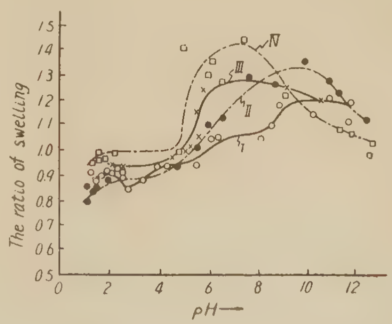


Fig. 13-4. The degree of swelling of *Stichopus japonicus* meat in solutions with or without salts of various pH values

As seen in curves I and III, the degree of the swelling of the meat is small in acidic side, but becomes larger from pH 5 to alkali side. The existence of neutral salt such as KCl increases the degree of swelling in the various pH solutions. Above pH 5.0 the existence of KCl increases especially remarkably the degree of the swelling, between pH 7 and pH 8 the swelling reaches the maximum. In alkali side the meat is peptized and a part of the protein dissolves out. On account of that dissolving the solution increases in turbidity and the degree of the swelling decreases. The same phenomenon was observed in curve II (without KCl) which shows the swelling in the solutions adjusted by N/25 HCl and N/25 NaOH solutions. In this case, the peptization is observed above pH 10. Curve II shows as a comparatively gentle-sloping curve for the change of the degree of the swelling in all range of pH ; the swelling has no remarkable change between pH 2 and pH 5. However, curve I showing the swelling in various pH solutions adjusted with N/25 H_2SO_4 and N/25 NaOH indicates complex and step-like changes. This is due to the isoelectric reaction of each protein (containing substitute protein) in the mixture of several proteins as mixture system[9].

Generally the swelling or the hydration of protein shows minimum degree at the isoelectic point, and increases to the acidic side or alkali side of the isoelectric point. In fact, Tarr,[36] Noguchi[9] and Okada[10] have studied the same subject using fish meat and Takahashi [32]and Lloyd[33] have studied the same subject using fish meat, collagen and fibroin. They have observed that the degree of the swelling of fish meat shows minimum value at the isoelectric point (pH 4~5) and the maximum value at alkali side (pH10~12) and acidic side (pH2~3), in the case that the fish meat is considered to be a mixture system. In the case of the coexistence of neutral salts, the swelling is accelerated in the alkali side, but it is inhibited in the acidic side as observed by Tarr[36] and Okada[10]. Comparing the swelling of fish meat with the results of observation of the swelling of *Stichopus japonicus* meat data on both which are shown in Fig. 13-4, the following two differences are remarkable. (1) On the acidic side, no maximum degree of the swelling of the meat of *Stichopus japonicus* is observed as may be seen in curve I and II(without addition of KCl). (In curve I, the degree of the swelling is observed to increase below pH 2.8, but it can not be called the maximum point). (2) In the case of curves III and IV showing the coexistence of KCl, the degree of the swelling is larger than that of curves I and II showing no addition of KCl. That is to say, the fact the existence of salts decreases the acid swelling of the meat of *Stichopus japonicus* was not observed. According to Okada[10]), the mechanism of the swelling of protein is different according to the kinds of acid, *e.g*, monovalent acid or bivalent acid. That is to say, HCl acts as a monobasic acid and H_2SO_4 acts as a dibasic acid, therefore, the swelling caused by HCl is larger than that caused by H_2SO_4. Comparing curve I with curve II of *Stichopus japonicus* meat below pH 5, there is no difference between them. Below pH 5 the swelling caused by H_2SO_4 is observed to be slightly

larger than that caused by HCl. On the contrary, in the case of coexistence of KCl, the swelling by HCl is larger than that caused by H_2SO_4.

As above stated, the swelling of *Stichopus japonicus* meat is remarkably different from that of fish meat (or fish skin). The cause of the difference are considered to be as follows: (1) The small amount of basic amino acid in the amino acid composition of *Stichopus japonicus* meat. (2) The larger amount of water content of *Stichopus japonicus* meat than that of fish meat; also the fact that the larger part of water in the meat of the former exists as free water in the network of the meat tissue. The free water is sealed as immobilized water[37]. When this immobilized water is oriented to free NH_2 radical in the acidic side, there is a definite limit to the amount of oriented water[38], therefore the larger part of the residual water including water from outside cannot take a part in hydration, and the swelling may not increase. Furthermore, (3) the properties of a kind of acid coagulable protein, a particular protein, may cause the difference. Among those assumed causes, those numbered (2) and (3) are considered to be the most probable causes. As to the assumption of difference (1) as a cause: *Stichopus japonicus* meat has almost the same amino acid composition, without lacking of basic amino acids, as fish meat as is also true in the nitrogen distribution of the fractionated proteins, which was explained in Article IV. Hence the cause of the difference (1) is considered to be proven incorrect. As to the assumption of difference (2) as a cause: a definite conclusion can not yet be obtained on account of insufficient data, but the characteristic of the meat of *Stichopus japonicus* having a larger amount of water content, about 90%, has intimate connection with the fact that the meat may swell with difficulty. As to the assumption of the causes of difference (3): the existence of an acid coagulated protein may have some intimate connection with the swelling and pH of the immersing solution. If the existence of that certain acid coagulable protein is responsible for a different swelling phenomenon in the fish meat in the acidic side, the facts that the swelling of the meat of *Stichopus japonicus* is accelerated by the addition of KCl even in acidic side or that there is no difference of the mechanism of the action of HCl or H_2SO_4 upon the meat protein of *Stichopus japonicus* from the viewpoint of the degree of the swelling, may be due to the increasing of the hydration upon the electrolytes of the acid coagulable protein or because of the characteristic construction of the meat.

From results as above obtained, it is difficult to determine the isoelectric point of *Stichopus japonicus* meat itself from the curve of the degree of the swelling as shown in Fig. 13–4, but it is desirable to determine the isoelectric point of each fractionated protein from the meat. As to this point, the isoelectric point of the meat protein of *Stichopus japonicus* is regarded to be at near pH 4.2 as stated in Article V. As seen in every curve in Fig. 13–4, the fact that the characteristic property differing from fish meat is observed in below pH 4.8 considered to reach the conclusion that the isoelectric point of the principal meat protein of *Stichopus japonicus* is pH 4–5.

XIV. HYDRATION OF THE MEAT AND MEAT PROTEIN OF *STICHOPUS JAPONICUS*

In order to ascertain the hydrating affinity of the meat and meat proteins of *Stichopus japonicus*, the author has determined the curves of the water-content — relative vapour pressure in the fresh meat, the water solubie protein, NaCl solution-soluble protein and NaOH solution-soluble protein which were fractionated from the meat. Comparisons are made with like values of other fish meat.

1. Hydration of the meat of *Stichopus japonicus*

(1) Experimental method

About 2 g of the crushed meat of *Stichopus japonicus* was accurately weighed, and this weighed meat was used for the sample. The vapour tension method[39] was used by an indirect procedure which estimates the equilibrium pressure between the vapour pressure of water contained in sample (P) and the vapour pressure of the pure water (dist. water) maintained at the same temperature as that of the sample by an oilmanometer. Thus each vapour pressure was measured for each different water content in the sample (dehydrated by vacuum drying). Estimations were made at temperatures of 18°±1°C. The results obtained are shown as the relative vapour pressure (p/p_0) to the amount of water content, "g", per gram of the dried matter.

(2) Experimental results



Fig. 14-1. "g-p/p_0" curves of *Stichopus japonicus* Atka mackerel and squid meat

Results obtained are shown as curve I in Fig. 14-1. In order to compare the meat of *Stichopus japonicus* with those of Atka mackerel and squid, the results obtained with those fish meats are shown as curves II and III of Fig. 14-1 respectively. Those results were obtained at 20°C.

As seen in Fig. 14-1, when the meat of *Stichopus japonicus* has about 0.3~0.4 of water content, "g", (gram of water per gram of the dried matter) (0.3~0.4 of "g" corresponds to about 23~29% of the water content for the sample containing water), the value of p/p_0 is 0.85~0.90. Below 0.3 of the value of "g", the value of p/p_0 decreases rapidly and shows as an S-type curve which is characteristic for the vapour pressure curve (curve of water content "g" — relative vapour pressure "p/p_0"). On the other hand, in the case of

Atka mackerel and squid meat, when the value of "g" is 0.3, the value of p/p_0 is 0.67 and 0.75 respectively, and the value of p/p_0 decreases rapidly with the decreasing of the value of "g". Observing from each curve in Fig. 14–1, the curve of *Stichopus japonicus* meat is situated farthest to the left side, the curve of squid is situated in the center, and the curve of Atka mackerel is situated most to the right. The water content "g" — relative vapour pressure "p/p_0" curve shown in Fig. 14–1 is regarded as a kind of dehydrating isothermal curve. The phenomenon of the mutual slipping of the curves is supposed to be owing to the difference of free energy of water in the sample, that is to say, to the difference of binding energy of water existing in the sample.

According to Lewis and Randall[40], the difference, $\Delta\bar{F}$, between the differential molal free energy of pure water and that of water in the sample at the same temperature is given by the following equation.

$$\Delta \bar{F} = F_0 - \bar{F} = - RT \ ln \ p/p_0 \ \ldots\ldots\ldots\ldots\ (1)$$

Here, F_0 is the differential molal free energy of pure water at T°K, $\bar{F}$ is that of water in the sample at the same temperature, and R is gas constant.

As seen in equation (1), in case the value of p/p_0 is constant, the value of $\Delta\bar{F}$ decreases with the increasing of the temperature, T°K, on the contrary, the value of $\Delta\bar{F}$ increases with the decreasing of the temperature. Therefore, in order to compare and discuss both curve I estimated at 18°C and curve II and III at 20°C, as a rule, it is desirable to obtain the curves estimated at the same temperature. However, if it be supposed that the value of $\Delta\bar{F}$ corresponding to any value of p/p_0 at 18°C (T=291°K) is calculated from equation (1), and that the value of "g" corresponding to above stated value of "p/p_0" is obtained from curve I of Fig. 14–1, and then that those values of $\Delta\bar{F}$ for those of water-content "g" do not change in even rising of temperature from 18°C to 20°C (T=293°K), then the value of p/p_0 should increase with the rising of the temperature, T. That is to say, the value of p/p_0 in the same amount of water content "g" increases with the rising of the temperature. Therefore, curve I′ showing "g" — p/p_0 which was supposed by the rising of the temperature from 18°C to 20°C slips as a whole more to the left side than curve I. Therefore, the difference between curve I′ which corresponds to 20°C estimation temperature becomes larger than that between curve I and curves II and III. As seen in Fig. 14–1, the result that curve I *of Stichopus japonicus* which was estimated at 18°C slips leftward (the value of p/p_0 slips to the direction of the value of 1 at the same amount of water content, "g") of curves II and III which were estimated in Atka mackerel and squid meat respectively at 20°C, means that the value of $\Delta\bar{F}$ of water in *Stichopus japonicus* meat is smaller than that of the other fish at every amount of water content "g". That is to say, the differential molal free energy ($\bar{F}$) of water in *Stichopus japonicus* meat comes up to the differential molal free energy ($\bar{F}_0$) of pure water, and this means that the binding energy

of water in the meat of *Stichopus japonicus* is small. As seen in curves I, II and III of Fig. 14–1, therefore, the binidng affinity of water in Atka mackerel meat is the strongest, while that of squid and *Stichopus japonicus* follow in order. This binding affinity is supposed to be due to the hydration with protein which is a principal component of the meat. The curve of "g" — p/p_0 for the sample changes also according to the concentration of the salts, but in this experiment, the concentration of the salts was out of the question, because the author desired to learn the binding affinity in the meat in natural state.

Consequently, it is considered that water in the meat of *Stichopus japonicus* is bound more loosely with protein (in the condition of the comparatively weaker affinity, even if the water molecules are able to orient to all of the hydrophilic groups contained in the protein molecules of the meat) than in the case of Atka mackerel and squid meat. Therefore, considering from the fact that *Stichopus japonicus* meat has larger amount of water content (about 90%) than fish meat and has a network of cnnective tissue, the most part of water is considered to be contained in the network and it is considered to be "immobilized" water.[37]

2. Hydration of the fractionated protein from the meat of *Stichopus japonicus*

Next, the curves of the water content "g" — relative vapour pressure "p/p_0" on the fractionated proteins from the meat of *Stichopus japonicus, viz.*, water soluble protein, 0.6 Mol NaCl solution-soluble protein, and 0.025 Mol NaOH solution-soluble protein, were determined by the vapour tension method.

(1) Experimental method

Put 1 g of each fractionated protein into about 10 cc of dist. water and inflate the protein at room temperature over night, and hydrate the protein. After centrifugation, the sediment, swollen protein, was employed for the experiment on the hydration. Each fractionated protein is not unique component, but is a complex of several components. The fractionated protein was prepared carefully, but a part of the protein denaturated. So the solubility of the protin in water became smaller. But the protein showed comparatively large swelling after immersion in water for a day. The swelling is supposed to be due to the hydration of the protein. NaCl solution-soluble protein and NaOH solution-soluble protein are primarily insoluble in water as seen from the treatment. If those proteins are dissolved in NaCl solution and NaOH solution respectively, they are mostly dissolved, but the vapour pressures of water in those proteins abnormally decreases owing to the existence of electrolytes, so it is difficult to compare the vapour pressures of water in those proteins with that in water soluble protein. In order to discuss the hydration of those proteins, water must be used as solvent in principle, even if those proteins are dissolved only slightly in water. The estimating temperature for water soluble protein was 15°±1°C, and for the other two proteins it was 19.5°±1°C.

(2) Experimental results

Results obtained are shown in Fig. 14–2.

Curve I of Fig. 14–2 shows water soluble protein, curve II shows NaCl solution-soluble protein and curve III shows NaOH solution-soluble protein. As seen in Fig. 14–2 curves II and III show almost the same tendency. From those curves the hydration of NaCl solution-soluble protein and NaOH solution-soluble protein are supposed to closely resemble each other. Comparing curve I showing water soluble protein with curves II and III at above 0.4 of the value of p/p_0, the water soluble protein seems smaller value of p/p_0 for the same amount of water content "g". Even if the water soluble protein swells for the same amount of water content to the same degree as the other two proteins at higher amount of water content, the former is supposed to have a stronger hydration than the latter two. At lower amount of water content below 0.4 of the value of p/p_0, curve II and curve III are almost the same. According to the adsorption theory of multimolecular layer offered by Brunauer et al.[41] the adsorbed amounts of water required to cover the surface of a unit quantity of those three kinds of proteins with a complete unimolecular water layer are supposed to be almost equal. Therefore it is supposed as a reason for water soluble protein having comparatively stronger affinity to water than the other two kinds of protein, that the molecular construction of water soluble protein presents more hydrating points (activating layer to water molecule) in unit quantity than the other two proteins. But having a large number of hydrating points does not necessarily mean a large number of hydrophilic groups in protein molecules. In salmine which has especially many hydrophilic groups, the adsorbed water has mostly small energy of adsorption (near the free state)[42], therefore the curve of "g—p/p_0" of salmine slips remarkably to the leftward.[43] It is expectable, therefore, that if the number of hydrophilic groups in the molecule of water soluble protein is small the molecular construction of the protein presents sufficient space where the water molecules are able to orient to the hydrophilic groups, and also that water molecules combine easily with the protein molecule. To compare the hydration of the fractionated protein from the meat of *Stichopus japonicus* with that of other fish meat[44], results obtained from NaCl solution-soluble protein of squid meat and NaOH solution-soluble protein of

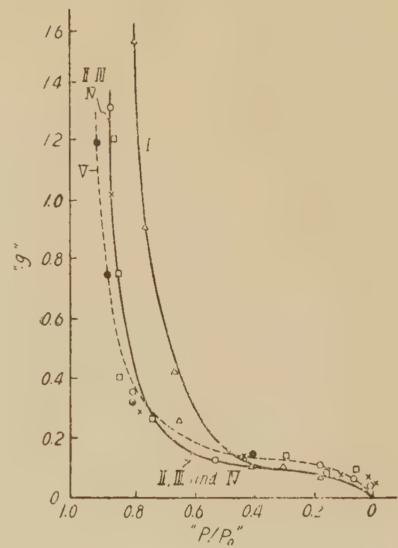


Fig. 14–2. "g-p/p$_0$" curves of the fractionated proteins from *Stichopus japonicus* meat

cod meat are shown as curve IV and V in Fig. 14–2, respectively.

Comparing curve II showing NaCl solution-soluble protein of the meat of *Stichopus japonicus* with curve IV showing that of squid meat the two curves show almost the same tendency, and are supposed to indicate similar hydrating affinity. But the temperature of NaCl solution-soluble protein of squid at which the estimation was made was lower than that of *Stichopus japonicus*. If the temperature of the estimation of NaCl solution-soluble protein of squid is 19.5° C, the same temperature as that of *Stichopus japonicus* meat, curve IV would probably slip more to the left side. That is to say, NaCl solution-soluble protein of squid meat has a molecular construction which hydrates more easily than that of *Stichopus japonicus* meat protein. In other words the former has a construction favorable to hydration predominant tendency to hydrate in the molecular arrangement or in the number of hydrophilic groups. Comparing curve III of the NaOH solution-soluble protein of *Stichopus japonicus* meat (at 19.5°C) with curve V of that of cod meat (at 15°C), in spite of the lower estimating temperature of curve V the latter slips more to the right than curve III. (Curve III shows larger value of p/p_0 than curve V at the same amount of water content "*g*"). That is to say, the meat protein of *Stichopus japonicus* has a stronger tendency to hydrate than that of cod meat protein. On the other hand the hydrating affinity of water in cod meat protein is stronger than that of *Stichopus japonicus* meat protein but the hydrating affinity is supposed to be comparatively weak.

Summarizing results obtained in the study of hydration using the vapour tension method the meat of *Stichopus japonicus* is more hydrophilic than fish meat. In the tissue of the former, there is contained a large amount of water. Most of the water is regarded as "immobilized free water" fixed in the network of the collagen fiber of the connective tissue. Among the proteins fractionated from the meat of *Stichopus japonicus* water soluble protein has a more hydrophilic molecular construction. On the other hand, the hydration of NaOH solubtion-soluble protein is inferior, but it has a very favorable molecular construction for comparatively strong binding with water.

XV. HYDRATION OF THE MEAT OF *STICHOPUS JAPONICUS* IN NaCl SOLUTION

From the results of experiments on the solubility of the meat protein of *Stichopus japonicus* in NaCl and KCl solutions, the solubility was known to be the greatest in about 0.6 Mol of the solutions (Article II). From the results of experiments on the swelling of the meat, the greatest swelling was known to be about 0.2 Mol in a monovalent salt solution (Article XIII). In the relation among the meat protein, water and salt ions, it is supposed that the hydrating affinity of the meat protein to water molecule is larger than that of salt ion at the concentrations from 0.2 to 0.6 Mol, and

at near 0.6 Mol, the both affinities reach equilibrium. Therefore the author has tried to clear the relation among them, meat protein, water and the concentration of NaCl solution, by employment of the vapour tension method for measuring the hydrophilic character of the present materials.

(1) Sample and experimental method

About 2 cm³ of meat blocks were taken from the bodies of *Stichopus japonicus* and weighed accurately. After the weighing, each meat block was put in 50 cc NaCl solutions having various concentrations (0, 0.2, 0.4, 0.6, 1, 2 Mol) and left at room temperature (20°±1°C) for 24 hours. After that period, a part of the meat block was used for the estimation of the amount of penetrated NaCl. Another part was used for the estimation of the amount of water content. The residual part of the meat blok was used for the estimation of the vapour pressures (p) (18°±1°C) corresponding to the various amounts of water content "g" in the same meat. The relative vapour pressures (p/p_0) corresponding to that of water content were calculated, where p_0 is the vapour pressure of the pure water (dist. water) at the same temperature as the meat.

(2) Experimental results

Table 15–1 shows the amounts of water content in sample meat and of sodium chloride penetraed into the sample. Results obtained are shown in Fig. 15–1 which

Table 15–1. The amount of water content in sample meat and of sodium chloride penetrated into the sample

Samples used	Water content in %	NaCl content in %
Meat immersed in dist. water	91.20	0.14
〃 〃 in 0.2Mol NaCl soln.	91.19	0.50
〃 〃 in 0.4Mol NaCl soln.	90.71	0.69
〃 〃 in 0.6Mol NaCl soln.	90.05	0.72
〃 〃 in 1.0Mol NaCl soln.	92.91	4.84
〃 〃 in 2.0Mol NaCl soln.	95.07	6.04

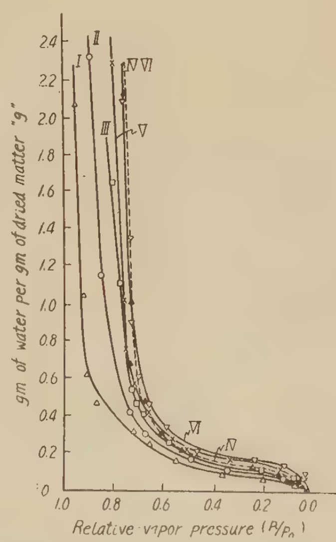


Fig. 15–1. "g–p/p_0" curves of *Stichopus japonicus* meat immersed in NaCl solution of various concentrations

shows the relation between the water content "g" of the sample and the relative vapour pressure "p/p_0" similarly to the relationships discussed above in Article XIV.

As seen in the curve of "g"—"p/p_0" of Fig. 15–1, below 0.6~0.7 of the value of p/p_0, the curves for samples immersed in water and 0.2, 0.4, 0.6, 1 and 2 Mol of NaCl

solutions are arranged in order. This fact proves the mutual relation between the amount of NaCl which penetrates into the meat protein and the hydration of the meat protein, *viz.*, that below 0.6~0.7 of the value of p/p_0, of which the water is regarded as bound water with protein[43][44], the greater amount of NaCl penetrated into the meat is, the greater the ion concentration becomes. Therefore, the hydrating affinity of salt ion increases and the dehydrating action of ion in respect to the bound water becomes larger. Consequently, as the amount of water which is regarded to be in the range of bound water decreases, the arrangement of the curves for the various concentration of NaCl solutions as shown in Fig. 15–1 is considered to come below 0.6~0.7 of the value of p/p_0. Above 0.6~0.7 of the value of p/p_0, the arrangement of the curves shows in the following order, water, 0.2, 0.4, 1, 2 Mol of NaCl solutions except 0.6 Mol NaCl. The particular result in the curve showing the immersion in 0.6 Mol NaCl solution may be explained by some discussion on the difference of binding abilities among NaCl ion, water and the protein molecule. Here, an attempt will be made to discuss the results obtained in these experiments. Firstly, the difference ($\Delta\bar{F}$) between the differential molal free energy of pure water and that of water in the sample of the block meat at the same temperature was calculated by Lewis and Randall's equation[40] (as shwon below) in order to discuss the particularity of the curve showing the immersion in 0.6 Mol NaCl solution. And then the curve of "$\Delta\bar{F}$ —g" (Fig. 15–2) was obtained.

$$\Delta\bar{F} = F_0 - \bar{F} = - RT\ ln\ p/p_0 \qquad (1)$$

Here, F_0 is the differential molal free energy of pure water at T°K, $\bar{F}$ is that of water in the sample at the same temperature, R is gas constant (1.985 cal.), T is absolute temperature (°K), p or p_0 is the vapour pressure of water in the sample or pure water at T°K respectively. In Fig. 15–2, the ordinate shows the value of $\Delta\bar{F}$, and the abscissa shows the amount of water content "g" which was obtained by the calibration from the value of "p/p_0" on the curves in Fig. 15–1, in which the value of p/p_0 corresponds to the value of $\Delta\bar{F}$ calculated from the above equation. As shown in Fig. 15–2, below 291 cal/Mol of the value of $\Delta\bar{F}$ corresponding to 0.6 of the value of p/p_0, the value of $\Delta\bar{F}$ for the same amount of water content, "g", increases in the order of the curves obtained from the immersing in water, 0.2, 0.4, 0.6, 1 and 2 Mol of NaCl solutions. Below 291 cal/Mol of the value of $\Delta\bar{F}$, in the case of the comparison at the same water content "g", the value of $\Delta\bar{F}$ on the curve showing the immersion in 0.6 Mol NaCl solution becomes larger than that on the other curves showing the

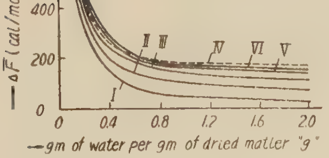


Fig. 15–2. "g-$\Delta\bar{F}$" curves of *Stichopus japonicus* meat immersed in various concentrations of NaCl solution

immersion in water, 0.2, ... 2.0 Mol NaCl solution with the increasing of the amount of water content, "g". This fact shows that the differential molal free energy of water in the meat immersed into 0.6 Mol NaCl solution increases. That is to say, it means the existence of a strong ability of protein to bind with water. Above 0.6 of the value of p/p_0 which is regarded as in the range of free water, it means that the solvation in 0.6 Mol NaCl solution, binding abilities among the protein, water and salt ions, is stronger than that in 0.2, 0.4, 1.0, 2.0 Mol NaCl solutions. These considerations are considered to support the experimental results on the solubility and the swelling. That is to say, the hydrating affinity of the meat of *Stichopus japonicus* immersed into 0.6 Mol NaCl solution is comparatively stronger than when it is immersed in other concentrations of NaCl solution.

XVI. HYDRATION AND SWELLING OF THE MEAT OF *STICHOPUS JAPONICUS* WHICH WAS BOILED AND DRIED TO VARIOUS DEGREES OF DEHYDRATION

The present author has previously studied on the swelling of dried squid meat ("Surume" in Japanese)[45]. At below 40% of total amount of water content of dried squid meat, the smaller the amount of water content is, the greater of the degree of the swelling shows. But, if the amount of dried matter during the swelling is taken into consideration, the same degree of swelling is observed below 40% of the total amount of water content of the dried squid meat. Takahashi[46] has also studied the absorption of water (swelling) by dried squid meat ("Surume"), and observed that in the range of 17~50% of the total amount of water content, the amounts of water absorbed in the dried squid meat are almost the same. The present author has observed the hydration and the swelling of the meat of *Stichopus japonicus* which was treated by boiling and then by drying as well as that prepared for canning.

(1) Experimental method

As samples, (A) fresh meat, (B) meat immersed in water for 24 houres, (C) meat cooked at 95°~99° for 15 minutes and (D) cooked meat which was dried under the sun to various degree of water content (48.3, 43.76, 21.63, 16.02 %) were employed. Among the four samples, (A) (B) and (C) were used for the estimation of curve of water content "g" — relative vapour pressure "p/p_0" at 18°±1°C by vapour tension method and for the estimation of the amount of bound water by cobaltous chloride method (Oyagi's method)[39]. In cobaltous chloride method, the amount of water estimated at the point at which the dyed pink sample immersed in 10 % cobaltous chloride solution turns to blue color during the drying below 30°C was regarded to be the bound water. (D)-Sample includes many samples having various amounts of water content. Each sample was immersed in dist. water for 24 hours and allowed to swell. The total amount of water in each sample and the degree of the swelling

($S = W/W_0$: "W" is the weight of the sample after the immersion in water, "W_0" is the weight before the immersion) were measured, and at the same time the curve of "g" — "p/p_0" was obtained at 18°±1°C by the vapour tension method.

(2) Experimental results

Results obtained with (A), (B) and (C)-samples are shown as curves I, II and III respectively in Fig. 16–1. Results with (D)-sample are shown as curves in Fig. 16–2. Curve III of Fig. 16–1 which shows the experimental result with sample (C) (meat cooked at 95°~99°C for 15 minutes) is rewritten in Fig. 16–2 for contrast as curve I.

The total amount of water content, the amount of bound water (g_{BP}), the degree

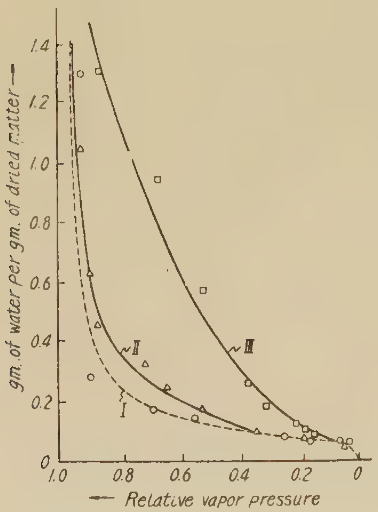


Fig. 16–1. "g-p/p_0" curves of raw meat, meat immersed in water and boiled meat of *Stichopus japonicus*

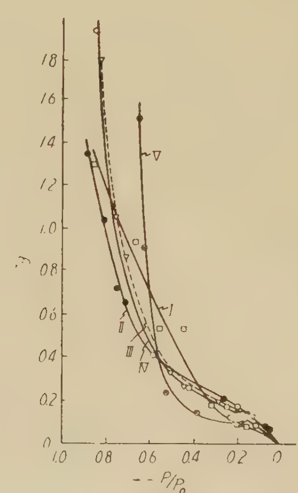


Fig. 16–2. "g-p/p_0" curves of boiled and dried meat of *Stichopus japonicus* swollen by immersion in water.

Table 16–1. The amount of total water, bound water content and the degree of swelling of *Stichopus japonicus* meat

Samples used	Total water content (%)	gm of water per gm of dried matter (g_{BP})	Degree of swelling (S)	Water content after swelling: Obs. value %	Water content after swelling: Calc. value %	Ratio of swelling (%)
Fresh meat	87.56	1.25				100
Boiled meat	80.86	0.763	0.595			
Immersed meat in H_2O	89.12	0.628	1.05, 0.97 } 1.01			(100)
Boiled & dried meat	48.30	0.693	1.41	85.11	63.2	34.1
Ditto	43.76	0.561	1.86	79.68	69.8	41.4
Ditto	21.63	0.507	2.67	72.45	90.7	42.7
Ditto	16.02	0.298	3.98	82.17	79.0	59.3

of the swelling "S", and the amount of water content after the swelling are shown in Table 16–1. In Table 16–1, the ratio of the swelling, "r", was calculated as follows. The fresh meat of *Stichopus japonicus* does not swell as a result of immersion in water. The degree of the swelling of the fresh meat after the immersing was 1.01 ($=S_0$). This degree of swelling is conveniently considered to be 1.0. Here, when 100 g of the fresh meat is dried to various degrees of the amount of water content, and these dried samples are immersed in water to swell, the ratio of the weight (gram) of the swollen meat to weight of the fresh meat, "r", is conveniently called the ratio of the swelling.

$$\text{Then, } r = S \times \left(\frac{D_0 W}{100-W} + D_0 \right) \quad \ldots\ldots\ldots\ldots (1)$$

where, "S" is the degree of the swelling ($S=W/W_0$), "r" is the ratio of the swelling, "W" is the amount of water content of the dried meat, and "p_0" is the weight of the dried matter in the fresh meat. Then, $D_0\%=100-W_0$ (W_0 is the percentage of water content in the fresh meat). In Fig. 16–1, comparing curve I (fresh meat) with curve II (meat immersed in water), above 0.5 of the value of the amount of water, "g", (33.3 % of the amount of water of the original sample) both the curves are shown as the same curve. Below 0.5 of "g", in the range of 0.88~0.85 of the value of p/p_0, the value of p/p_0 of the meat immersed in water (Sample B) is smaller than that of the fresh meat (Sample A) for the same amount of water content, "g". Curve III showing the boiled meat is found to right side of curve I showing the fresh meat, therefore, the value of "p/p_0" of the boiled meat is smaller than that of fresh meat for the same amount of water content, "g". As explained in the previous Article XIV on the hydration of meat and meat proteins of *Stichopus japonicus*, the greater the value of ΔF is, the smaller the value of p/p^0 is, for the same amount of water and the same temperature. This fact is due to the increase in the binding ability of the protein, a principal component of the meat, with water in the sample. Consequently, the hydrating affinity of the meat immersed in water is considered to be stronger than that of the fresh meat. But, the space between curves I and II is considered to be insignificant, since a part of the water soluble protein or other soluble compounds in the meat immersed in water is dissolved out.

Generally speaking, the meat of *Stichopus japonicus* does not absorb water at room temperature, that is to say, the value of the degree of the swelling of the meat immersed in water is 1.01 on the average, as seen in Table 16–1. Frequently the value becomes below 1. Therefore, the fact that the range of higher amounts of water, the curves of the fresh meat and the meat immersed in water are the same, is considered to be characteristic to the meat of *Stichopus japonicus* in comparison with the fish meat. The fact that the value of ΔF of water in the cooked meat of *Stichopus japonicus* is larger than that of fresh meat is considered to be due to heat denaturation, the removing of mineral salts or soluble protein. Just like fish meat, the meat of *Stichopus*

japonicus lets loose the free water as a result of cooking. As seen in Table 16–1, the amount of water content is decreased from 87.5 % to 80.86 % by cooking, and the remaining water is considered to be strongly bound with protein. As shown in Table 16–1 the value of the amount of bound water (g_{Bp}) of the fresh meat decreases from 1.25 to 0.763 per gram of the dried matter by cooking, that is to say, the approximate amount of the bound water decreases by cooking, but the binding ability is considered to be strengthened. The value of the amount of bound water (g_{Bp}) in the meat of *Stichopus japonicus* is 1.25. This value is larger than that of fish meat which is 0.5~0.8[47]. The hydrating affinity of the meat of the former is smaller than that of fish meat as stated in Article XIV, therefore, the higher value of the amount of bound water (g_{Bp}) is considered to be due to the particular construction of the meat, the network construction. Results obtained to sample D (the dried meat samples having various degrees of water) which is swollen in water, are shown as curve II (the total amount of water is 48.3%), curve III (43.76%), curve IV (21.63%) and curve V (16.02%). Those curves cross complexedly in the range of high amount of water or low amount of water, but as a general tendency, the curves show more or less uneven in comparison with curve I (the cooked meat) as standard. From those results, the hydrating affinity of Sample D (after cooking, meat dried to various degrees of water content) is considered to be almost the same as that of the cooked meat.

As to the variation of the amount of bound water in the meat of *Stichopus japonicus* meat during the drying, as seen in Table 16–1, with the decreasing of the total amount of water content in the meat, the value of the amount of bound water becomes small. Therefore, denaturation of the meat protein occurs, and consequently increase in the hydrating affinity is expected. That is to say, in Fig. 16–2, above 0.4 of the amount of water, "g", the curves II, III and IV slip to the right with the decreasing of the total amount of water (the value of p/p_0 decreases at the same amount of water content). From those results, the increasing of the value of ΔF of water in the sample is observed, which is added evidence of the increasing of the hydrating affinity.

Considering from the relation of the hydration and the swelling, as seen in Table 16–1, the degree of the swelling, "S" becomes larger with the smaller amount of water in the dried meat. From equation (1), if "r_0" is the ratio of the swelling and "W_0" is the amount of water content in fresh meat of *Stichopus japonicus*, from $D_0=100-W_0$, $D_0+W_0=100$, therefore,

$$r_0 = S_0 \times \left(\frac{D_0 W_0}{100-W_0} + D_0\right) = 100\, S_0$$

where, S_0 is the degree of the swelling of the fresh meat immersed in water. As "S_0" was supposed to be 1.0, then $r_0=100\times 1.0=100$. That is to say, when 100 g of the fresh meat of *Stichopus japonicus* is immersed in water, the weight of the meat is unchanged. Then the ratio of "r" which is the ratio of swelling of dried meat to

"r_0", the ratio of swelling of fresh meat, is shown as the following equation.

$$r/r_0 \times 100 = \frac{S}{S_0}\left\{\frac{D_0 W}{100-W} + D_0\right\} = S \times \left\{\frac{D_0 W}{100-W} + D_0\right\} \quad \ldots\ (2)$$

This equation shows the ratio of the degree of the swelling of the dried meat to that of the fresh meat immersed in water. As the fresh meat has 87.54 % water content, D_0 is 100−87.54=12.46%. The amount of water content and the value of the degree of the swelling of each dried meat having various degrees of drying were actually measured. Those values were substituted in equation (2), and the calculated values are shown in the last column of Table 16–1. As seen in that column, for example, if the dried meat of *Stichopus japonicus* having 48.30% of water content is allowed to swell in water, the dried meat shows 34.1% of the swelling degree of that of fresh meat immersed in water. The value of "r" increases with the decrease of water content in the dried meat ; at 16% water content in the dried meat, the value of "r" is 59.3%. Below 50% of the total amount of water content in the meat of *Stichopus japonicus*, the following result is apparently observed — the smaller the amount of water content is, the larger the degree of the swelling. This results is also observed in the case of dried squid meat* ("Surume" in Japanese). Here, the loss of the dried matter from the dried meat of *Stichopus japonicus* must be considered. If there is supposed to be no loss of the dried matter, the amount of water content "W_s" in the meat after swelling should be calculable theoretically by the substitution of the known values of the amount of water content "W" and the degree of swelling "S" into the following equation.

$$W_s = \frac{100\,(S-1) + W}{S} \quad \ldots\ldots\ldots\ldots\ (3)$$

The values of "W_s" calculated are shown in the 6th column of Table 16–1. From those values, it is evident that the amount of water content increased relatively according to the loss of the dried matter. The slipping of curves II, III, IV, V in Fig. 16–2 from curve I is considered to be due to the loss of the dried matter and it means an increase in the hydrating affinity of the remaining protein.

Summarizing from results obtained, if the fresh meat of *Stichopus japonicus* is immersed in water, no phenomenon of swelling is observed, and there is no difference in respect to hydrating affinity between the fresh meat (Sample-A) and the meat immersed in water (Sample-B). But if the boiled and dried meat is allowed to swell by immersion in water, shows about 40% of the ratio of the degree of swelling to that of the fresh meat.

* In the range of 49~23 % total water content, the dried squid "Surume" shows the "r" value of 62~72 %, so the boiled and dried *Stichopus japonicus* meat shows clearly smaller degree of swelling than the dried squid meat[45].

XVII. ON THE MUCOPROTEIN IN THE MEAT OF *STICHOPUS JAPONICUS*

It is unknown at the present time just what the chemical mechanism may be which would explain the phenomenon of the remarkable mucosity in the meat of *Stichopus japonicus*. It might be due to a gland secretion similar to that in the snail or due to some deforming substance which was formed by a particular chemical action or by an enzymic action. Although the chemical mechanism is unknown, the mucous substance clearly has formed collagen fibers (Article VIII). Perhaps, the collagen fibers combined with polysaccharides are changed by gelatinization to form a mucous substance. When a small quantity of the mucous substance was tested by Biurett reaction, Xanthoprotein reaction, Millon reaction, Sakaguchi reaction, and diazo reaction, all the reactions showed positive. That is to say, the presence of the protein was definitely and certainly observed. The presence of free sugars, amino sugars and uronic acid was likewise ascertained by Molish reaction and naphthoresorcinol reaction. Therefore, the mucous substance was supposed to be mucoprotein. Here, the author has tried to clarify the properties and the composition of the mucoprotein.

1. Preparation of mucoprotein from the meat of *Stichopus japonicus*

As to the general properties of the mucoprotein, it has high solubility in water and salts solutions, and has high stability against the reagents for many proteins except those for itself. For the preparation of the mucoprotein from the meat of *Stichopus japonicus*, after the meat tissue and the mucous substance were extracted with water, the mucoprotein was precipitated by means of the addition of HCl solution[48].

The eviscerated bodies of *Stichopus japonicus* were left at room temperature (about 20°C). Then a large amount of the mucous substance was formed. Add five times volume of alcohol to the mucous substance and precipitate the changeable substance in the mucous substance. After removing the changeable substance by filtration, extract the filtrate repeatedly with three times volumes of dist. water at 40°C during 5 days standing. Filter the brown colored extract first with silk cloth and then with filter paper. Extract repeatedly with water the remaining stroma left after silk cloth and filter paper filtration. As the filtrate is about pH 6.0, it is neutralized with 0.05 N NH_4OH. Add gradually dil. HCl solution to the neutralized filtrate to make the solution contain about 0.2% of HCl, and to bring the pH value of the solution below 1.4. The protein in the filtrate is precipitated by addition of HCl, and the remaining protein in the filtrate is precipitated completely by heating on boiling bath. Filter this brown colored solution again with a filter paper. After the filtration, the filtrate is once more neutralized with 0.05 N NH_4OH and acidified with 0.2% HCl solution. At this time, the larger sediment is formed at pH 3.4. Wash this sediment with alcohol and ether repeatedly and a colorless sediment is obtained. Leave this sediment in a brown vacuum desiccator containing phosphorus pentoxide. The dried

sediment was obtained to the amount of about 0.13% of the original substance (about 0.95% of the dried matter). Hisamura[49] has prepared mucoprotein from the dried nasal septum and bull trachea by the same method as the author's preparation, the yield was 0.97% for nasal septum of whale and 0.48% for bull trachea. The yield of mucoprotein of the meat of *Stichopus japonicus* was nearly in agreement with that of the nasal septum of whale.

2. Qualitative reactions of the isolated mucoprotein

(1) Qualitative reactions of protein, amino sugar and uronic acid

As described below, the protein, amino sugar and uronic acid in the isolated mucoprotein were tested by qualitative reactions. In protein reaction, Biurett reaction gave violet, Sakaguchi reaction gave red, and diazo reaction gave orange-red color. Molish reaction for amino sugar in the protein show green in the lower layer of the test tube, red in the upper layer. This protein was precipitated by trichloracetic acid or phosphotungstic acid which was acidified with H_2SO_4, but not precipitated by mercuric chloride, and not precipitated by heating. The naphthoresorcinol and anilin acetate reactions for uronic acid were postive. As above shown, the mucoprotein prepared is considered to contain amino sugar, uronic acid.

(2) Detection of carbohydrates moiety in the isolated mucoprotein

Next, the carbohydrate moiety in the mucoprotein was detected by paper chromatography. There are many investigations for sugars[50]; the present study was done by the following method.

(i) Preparation of the sample for paper chromatography[51]

Heat 0.1 g of the isolated mucoprotein with 3 cc of 1 N H_2SO_4 at 100°C. After hydrolysis, add baryta to the hydrolysate to make its pH near 5.8, and centrifuge the solution. After the centrifugation, add baryta to the upper clear liquor to make its pH 5.8. After 2nd centrifugation, the upper clear liquor was used for the paper chromatography.

(ii) Developing solvents and revealing reagent[52]

As developing solvents, 1 % ammonium containing phenol and a mixture of butanol, acetic acid and water (4 : 1 : 5) were used. The developing method was by two-dimensional chromatography by ascending solvent. For general sugars, ammoniac silver nitrate solution was sprayed and sprayed paper was heated at 100°-105°C, whereupon the developed materials were revealed. This method is suitable to reveal the spots of reductive derivatives such as methyl sugar, ascorbic acid and those of non-reductive derivatives such as saccharose and glucosid. Therefore, if it is employed to reveal only the spots of sugar, it will not be complete. But, there are some differences in the course of revealing, *e.g.*, the reductive sugar is revealed rapidly and non-reductive sugar is revealed slowly as brown or dark brown spots. Here, the author has observed that the suitable heating time is 5 minutes at 100°-105°C after tests to measure the

time until the revealing, by employing various concentrations of pure dextrose. The revealing of the spots of pentose and uronic acid is observed, by means of a mixed solution of alcoholic solution containing 1 % naphthoresorcinol and 1 % trichloracetic acid, as blue spots in the presence of moisture[53]. Therefore, as revealing reagents for the spots of carbohydrate which were detected by a parallel experiment, ammoniac silver nitrate solution and the mixed solution above described were used with each other. For the detection of amino sugar N-acetylify the sample with a mixed solution of acetylaceton and n-butanol then spray with Ehrlich's reagent and at last reveal the spots by heating at 90°C for 5 minutes. In this case, if the amino sugar is present naturally as an acetyl detivative it has a different position of spot from the acetylated amino sugar which was acetylated by the foregoing treatment. By the above described chromatographic method galactose N-acetylglucosamine, N-acetylgalactosamine and glucuronic acid were detected. Free sugar detected was galactose only. But except galactose, other pentoses or hexoses which are abundantly present in the living body were not observed in the chromatograms which shows only the presence of glucuronic acids. Amino sugars was also detected to be present as N-acetyl-derivative. Other free amino sugars were not detected.

3. Quantitative estimation of mucoprotein in the meat of *Stichopus japonicus*

(1) Quantitative analysis

(i) The total amount of nitrogen was estimated by the micro Kjeldahl method. (ii) Acid-hydrolyzable sulfur was estimated gravimetrically as $BaSO_4$ after hydrolysis by 1 N HCl solution. (iii) The amount of glucuronic acid was estimated by Tanabe[54]. Put about 0.1 g of the isolated mucoprotein into a decomposing bottle of 250 cc volume, and add 20 cc of conc. HCl (sp. gr. 1.19) to the mucoprotein. Heat the bottle with reflux condenser. After cooling, pour 10 cc of dist. water into the bottle. Then neutralize the hydrolysate with solid sodium carbonate to litmus paper. Then steam distil the neutralized solution. The distillate was back titrated with 0.01 N sodium thiosulfate. (iv) The estimation of reducing power. Heat about 10 mg of the isolated mucoprotein with 8 cc of 1 N HCl in a decomposing bottle attached to reflux condenser for 4 hours. After hydrolysis, neutralize the hydrolysate with 2 cc of 4 N NaOH. Add dist. water to she neutralized solution to make 12 cc in total volume. After the addition of 0.005 N potassium ferricyanide, the reducing power of the solution was measured by Fujita's method[55]. (v) The estimation of the amount of galactose was done by Hagedorn-Jensen's method which was introduced by Gale[56]. (vi) The estimation of acetyl was done by Friedrich, Rapapport and Sternberg's method which was reformed by Suzuki[57]. (vii) The estimation of the amount of amino sugar was done by colorimetry which was introduced by Masamune and Nagazumi[58].

(2) Results

Results obtained are shown in Table 17–1 in which the results obtained from the

nasal septum of whale and bull trachea[49] are presented in company with those of *Stichopus japonicus*.

Table 17-1. Comparative results on mucoprotein prepared from *Stichopus japonicus* meat, nasal septum of whale and bull trachea

Item	*Stichopus japonicus*		Nasal septum of whale (2)	Bull trachea
	In per cent	In equivalent per E.W.	In per cent	In per cent
Nitrogen	13.03		12.15	12.49
Acid hydrolyzable sulfur	1.27	0.98	1.81	1.73
Amino sugar	15.66	2.00	14.7	14.0
Galactose	7.81	1.05	9.3	7.1
Glucuronic acid	7.83	0.92	6.06	6.98
Acetyl	4.83	2.50	3.55	—
Total reduction	25.75		24.4	22.7
Ash	1.08		0.81	0.65

As seen in Table 17–1, in the mucoprotein isolated from the meat of *Stichopus japonicus* the amount of acid-hydrolyzable sulfur is obtained to be somewhat smaller than that in the other mucoprotein. This equivalent value, however, from the mucoprotein in *Stichopus japonicus*, is rather in agreement with the theoretical value, and that from the other mucoprotein is considered to be excessive. Suzuki[57] and Hisamura[49] have explained this fact by suggesting that mineral impurities mixed into mucoprotein at the preparation of tendomucoid and chondromucoid may have caused the disagreement. As the equivalent proportion of acetyl and amino sugar is $4.38/43 : 15.66/179 \doteqdot 1$ in the isolated mucoprotein, then the amino sugar is considered to be present as an acetyl derivative in the mucoprotein in the meat of *Stichopus japonicus*. As the equivalent ratio of galactose and galactronic acid is about 1 : 1, the equivalent proportion of acetylhexosamine and galactose is about 2 : 1. As the equivalent value of acid-hydrolyzable sulfur is 0.98, this sulfur is considered to combine as a molecule of itinsulfuric acid such as chondroitinsulfuric acid. In the molecule of itinsulfuric acid, as uronic acid and hexosamine sulfuric acid are combined in equal volume, amino sugar in Table 17–1 must be divided into two groups.

From these considerations the existence is supposed of a polysaccharide in which the equivalent ratio of acetylhexosamine and galactose is 1 : 1 as a unit sugar composition. Consequently, the polysaccharide combined with meat protein consists of two groups of acidic mucopolysaccharide such as itinsulfuric acid containing glucuronic acid and of neutral mucosaccharide which contains acetylhexosamine and galactose in equal quantity. Both the acidic and the neutral mucopolysaccharide are considered to be united with the protein moiety in the isolated mucoprotein at equimolecular ratio. As seen in Table 17–1, the amounts of gucuronic acid in the mucoprotein of the meat of *Stichopus japonicus* show somewhat low values. As

Suzuki has observed that the amount of furfural formed in glucuronic acid combined in itinsulfuric acid is generally smaller than glucuronic acid in the free state, this lower value is questionable as it may be due to experimental error. The result for acetyl and the value of the amount of ash are considered to be due to experimental errors.

4. Isolation of the unit of the polysaccharide moiety in the mucoprotein

As a prosthetic polysaccharide in the mucoprotein in the meat of *Stichopus japonicus*, the presence was supposed of two groups, one an acidic mucopolysaccharide such as itinsulfuric acid, and the orther a neutral mucopolysaccharide which contains hexosamine and galactose as the unit sugar composition. In order to determine the composition of each polysaccharide, the author fractionated both the prosthetic polysaccharides. The fractionaion was carried out by Hisamura's method[49] as according to the following Scheme 17–1. Fraction A in Scheme 17–1 was treated according to Scheme 17–2, and the purification was repeated.

(1) Qualitative detection of A-fraction

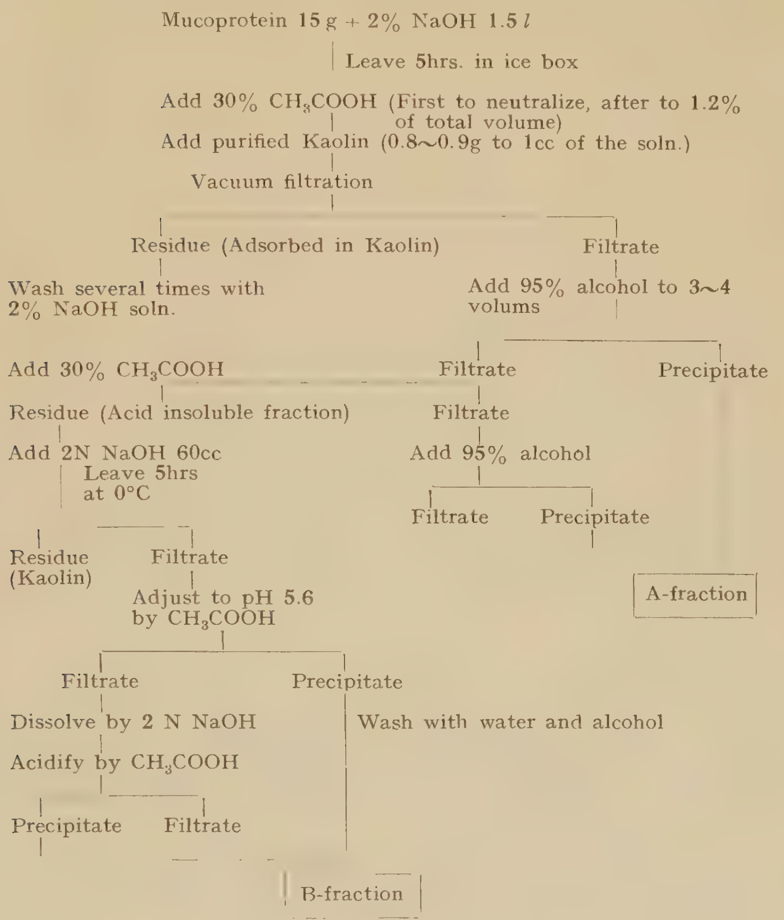
The substance obtained in A-fraction was in the form of white amorphous crystals, of which the estimation of the melting point and the quantitative analysis could not be made, because of the small amount of the yield. Some of the solution in which the crystals dissolved was tested by Biurett reaction which resulted negative. It showed remarkably green by Molish reaction. It was also turned slight red by Ehrlich's reagent. It showed no flow birefringence. The chemical composition of the substance in A-fraction was determined by chromatography with the use of the solution left after hydrolysis. The chromatography was carried in the same manner as described in the qualitative analysis of mucoprotein.

Here, the chromatography was made by the one-dimensional ascending method. As the developing reagent, 1% ammoniac phenol was used. As the revealing reagent, ammoniac silver nitrate was sprayed for the detection of free sugar and reducing substance, and for the detection of free amino sugar, N-acetylated sample with acetylation was tested by Ehrlich's reagent. For the detection of uronic acid, naphthoresorcinol and trichloracetic acid were used. In A-fraction only acetylgalactosamine and glucuronic acid were detected by the combination of various revealing reagents. Considering from the results of qualitative test, as Masamune[58] pointed out, the isolated substances in the A-fraction were itinsulfuric acid, closely similar to chondroitinsulfuric acid. Although Blix et al.[59] stated that chondroitinsulfuric acid ester shows flow birefringence, the observations of this point using the isolated substance in the experiment were negative. The reason for such a fact raises a question. This is, however, considered to be becauses the isolated itinsulfuric acid may form so-called sub-unit or the amount of the solute in the water solution may be too small.

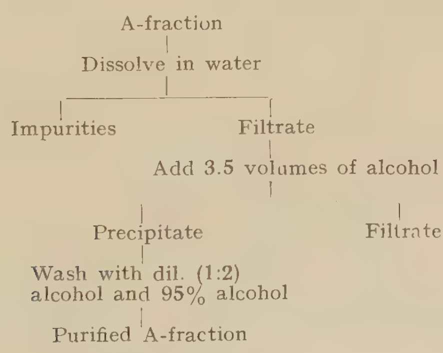
(2) Qualitative detection of B-fraction

Scheme 17-1.

Preparing procedure of prosthetic polysaccharide from mucoprotein



Scheme 17-2. Purification of A-fraction from mucoprotein

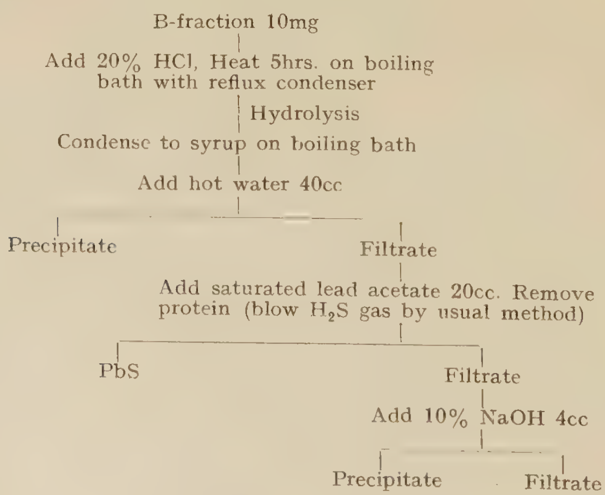


The isolated substances in B-fraction were purified before the qualitative detection. Dissolve the sample in NaOH solution of pH 9, and centrifuge the solution to remove the suspended impurities. Add 30% acetic acid solution to the upper clear liquor to bring pH below 4. Then sediments are obtained. After filtration, wash the sediment with 95% alchohol repeatedly. Dry the washed sediment in a brown colored dessicator containing phosphorus pentoxide. Dissolve the sample thus purified in water. After hydrolysis of this solution, the chemical composition of the hydrolysate was detected by chromatography as in A-fraction. The substances in B-fraction detected by chromatography are galactose and acetylglucosamine. As already suggested from the quantitative result the examination of carbohydrate moiety in mucoprotein, the prosthetic polysaccharides are constituted of two sorts of components. The components in each fraction as obtained by alkali fractionation are qualitatively clarified in this experiment. In A-fraction, the two kinds of polysaccharides fractionated were acetylgalactosamine as hexosamine and itinsulfuric acid containing glucuronic acid which is as uronic acid, and having properties similar to those of chondroitinsulfuric acid. In B-fraction, galactose as free sugar and acetylgalactosamine, glucuronic acid exist as unit constituents of sugar. Those components combine in equimolecular ratio. Another polysaccharide consists of galactose and acetylglucosamine as unit composition of sugar, of which the ratio of composition is considered to be 1 : 1.

5. Fractionation of amino sugar

The amino sugar was fractionated from the polysaccharide in B-fraction according to the following scheme. The yield of the sediment obtained was about 3 mg in average. This sediment shows red color when Ehrlich's reagent is added. Dissolve

Scheme 17-3. Isolation of amino sugar from B fraction



this sediment in acid solution with HCl to make the sample for paper chromatography as described below. The components of this sample were detected by chromatography to be accompanied with pure glucosamine hydrochloride and N-acetylglucosamine which was prepared by acetylation from the pure glucosamine. Chromatography was carried out by one-dimentional method developing with 1% ammonium containing phenol, and revealing with Ehlrich's reagent. Each respective values of Rf for the sample pure, glucosamine and N-acetylglucosamine were 0.71, 0.64 and 0.70. Therefore the amino sugar in the sample is considered to be N-acetylglucosamine.

6. Nitrogen distribution of the protein moiety in the mucoprotein

The mucoprotein prepared by treatment previously described was hydrolyzed with 20% HCl solution. After the hydrolysis, the nitrogen distribution was determined by Van Slyke's method[4]. The results obtained are shown in Table 17–2.

As seen in Table 17–2, about 27% of the total amount of nitrogen in the mucoprotein is basic amino acid nitrogen while 59% is mono amino acid nitrogen. Comparing those values with the fractionated proteins, which are detailed in Article IV, the distribution of nitrogen of the mucoprotein was observed to be almost the same as that of fractionated proteins, particularly like water soluble protein.

Table 17–2. Nitrogen distribution of mucoprotein isolated from *Stichopus japonicus* meat

Fraction	in sample (%)	in total-N (%)
Total-N	6.89	100
Amide-N	0.29	4.21
Humine-N	0.46	6.68
Total-N in form of bases	1.84	26.7
Amino-N in form of bases	1.12	16.3
Total-N in form of mono amino fraction	4.03	58.5
Amino-N in form of mono amino fraction	2.88	43.2
Non-amino-N in form of mono amino fraction	1.15	16.7
Sum	6.62	96.09

7. Experiments on the electrophoresis of the mucoprotein which was fractionated from the meat of *stichopus japonicus*

In order to ascertain the composition of the protein moiety of the mucoprotein, the author undertook to observe the electrophoretic patterns.

(1) Experimental method

Add the mixture of 95% alcohol and water (1 : 1), five times in volume, to the meat of *Stichopus japonicus*, leave the mixture at 40°C after 5 days. Adjust the extract to pH 7.0 with 0.05% ammoniac solution. Add conc. HCl to the solution to make the value of pH 1.4. Heat this solution on a water bath. Centrifuge the sediment formed during the heating. Extract this sediment of protein with 0.05% ammoniac solution. Remove the color substance from the ammoniac solution con-

taining the protein with a mixed solution of ether and alcohol. After the removal of the layer containing color substance, the water layer is obtained. When HCl is added to the water layer, then a sediment of protein is formed. The protein thus obtained by repeating the treatment twice was used for the electrophorsis observation. Dissolve about 4 g of the wet matter of the protein into the phosphate buffer solution (pH 8.1) of which the ionic strength, μ, is 0.15 to make 20 cc of total volume, and leave the solution over night. The protein was completely dissolved. Dialyze the protein solution in 500 cc of the same buffer solution of 0.15 ionic strength for 48 hours (at 4°~8°C). After dialysis, the protein solution was centrifuged. After the centrifugation, the upper liquor was used as the sample for the electrophoretic experiment under the conditions of potential grade 5.75 volt/cm, and 7.9 mA of electric current. The apparatus used was HT type Tiselius. The size of the cell was 2×15×150 mm. The temperature during experiment was held at 4°~6°C.

(2) Experimental results

Results obtained are shown in Table 17–3 and Fig. 17–1.

Table 17–3 Protein components and the mobilities of mucoprotein isolated from *Stichopus japonicus* meat

Components		Mobility (cm^2/volt. sec.)	Remarks
Comp. I	Des.	-19.8×10^{-5}	$\mu=0.15$
	As.	19.4	5.75 volt/cm
Comp. II	Des.	−7.1	pH 6.71
	As.	11.3	

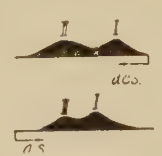


Fig. 17–1. Electrophoretic pattern of mucoprotein isolated from *Stichopus japonicus* meat (descending and ascending boundary)

As seen in Fig. 17–1 the mucoprotein which was prepared as above stated has two components of which the mobilities are 19.4 and 11.3×10^{-5} cm^2/volt. sec. by ascending limb. Component II has similar mobility to component III which has already been found in the water soluble protein fraction of meat of *Stichopus japonicus* (Articles III, V). But it is questionable whether the two proteins are the same or not. Consequently, it became clear that the protein moiety in the mucoprotein of the meat of *Stichopus japonicus* consists of two components, between the mobilities of which there is a remarkable difference.

XVIII GENERAL DISCUSSION

The author has studied the characteristics of meat protein of *Stichopus japonicus*, and obtained new items of knowledge about it. The results obtained may be summarized as follows.

(1) A greater amount of water is contained in *Stichopus japonicus* than in fish

meat. (2) The meat is easily and naturally deformed in the air after the catch, that is to say, it is easily softened. (3) The meat is difficult to dissolve in water. (4) The fresh meat is difficult to dry. (5) When the meat is cooked, it contracts remarkably, and if one push the cooked meat with his finger, it is easily broken. (6) When the meat is boiled and dried, it is difficult to tear in any direction either horizontally or vertically. (7) A smaller total amount of nitrogen is contained in *Stichopus japonicus* meat than in fish meat. (8) The isoelectric point of the meat is near pH 4.2, and it is more to the acidic side than that of fish meat. (9) If the meat is repeatedly extracted by water, the aqueous extract begins to show flow birefringence as well as squid meat[3] after the 3rd or 4th extraction. (10) The meat is contracted by heating with almost the same ratio in all directions horizontally and vertically. (11) The meat coagulated with acids ; it is difficult to gelatinize in the acid solutions of low pH values. (12) The fresh meat does not swell esaily in water or in salt solutions. (13) The hydrating affinity of the meat is weak. (14) The meat contains mucoprotein.

Those results are discussed and explained as follows.

1. A greater amount of water is contained in the meat of *Stichopus japonicus* than in fish meat.

The chemical composition of the meat of *Stichopus japonicus* more or less varies with the seasons. In that composition, the variation of the amount of water content is most remarkable. The amount of water content is 86~92% throughout the year ; it is greater than that of fish meat which shows 75~85%.[1] The edible part (body wall) of the body of *Stichopus japonicus* is histologically not muscular tissue, but consists mainly of connective tissue, composing a network of collagen fibers (Article I). In fact, the amount of collagen in the meat protein of *Stichopus japonicus* is about three times that in fish meat (Article X). In the network of collagen fibers there is contained body fluid containing soluble proteins *e.g.* myosin and myogen (Articles III, XII). The hydrating affinity of the meat is inferior to that in fish meat (Article XIV). About 82% of the total amount of water content is "immobilized water" having properties of free water[37]. Therefore, when the body of *Stichopus japonicus* is left in a bamboo basket, the greater part of the water flows out from the body.

2. The meat is easily and naturally deformed in the air after the catch, that is to say, it is easily softened.

When the bodies of *Stichopus japonicus* are left alone in the air, the body deforms, and it loosens from the surface to cause the inner meat to become mucous[2]. At this time, if the body is touched with a finger or with a straw, the body is observed to loosen naturally at a faster rate. This phenomenon seemed to be enzymic chemical in its nature. Besides this possible explanation, the phenomenon may be explained

as follows. The "immobilized water" fixed in the network of the connective tissue increases its thermodynamic energy with the rising of the environmental temperature, accompanying with the denaturation of protein, therefore, the tissue is broken and the body fluid flows out. Then the meat becomes mucous.

3. The meat is difficult to dissolve in water.

Squid meat is composed from a more water soluble protein than that of fish meat[8]. *Stichopus japonicus* meat is composed mainly of insoluble protein, *viz.*, collagen; it is considered to dissolve with difficulty in water. But there is some amount of soluble protein in the network of the connetive tissue, because the water soluble protein, NaCl solution-soluble protein and NaOH solution-soluble protein were fractionated (Article III).

4. The fresh meat of *Stichopus japonicus* is difficult to dry.

The hydrating affinity of the meat is comparatively weak (Article XIV), but the meat is difficult to dry. (This difficulty varies according to the drying temperature, here the drying temperature is considered to be "natural drying" or below 35°C).

A cause of this phenomenon is considered to be the presence of "immobilized water" fixed in the network of the collagen fibers (Article XIV).

5. When the meat of *Stichopus japonicus* is cooked, it contracts remarkably, and if one pushes with a finger on the cooked meat, it is easily broken.

When the meat is cooked, it contracts rapidly at near 40°C, and it shows a contraction of about 50% at temperatures above 70°C (Article IX). If one pushes with a finger on the cooked meat, it is easily broken. The appearance of breaking is similar to that of the gelatine gel which slits in all directions horizontally and vertically. Those facts are considered to be due to the facts that the meat consists of connective tissue composing a network of collagen fibers and a part of the body fluid in the network flows out by the contraction of the collagen fibers (or by the gelatinization of the collagen fibers) during the cooking. The network of collagen fiber in the connective tissue consists of anisotropic particles, which are submicroscopic rodlets, arranged parallel to the longitudinal axis of the fiber[60], therefore the meat is considered to be difficult to tear in one direction (Article VIII).

6. When the meat is boiled and dried, it is difficult to tear in any direction, horizontally or vertically.

If the cooked meat of *Stichopus japonicus* is dried in the air, the meat becomes elastic, and becomes difficult to tear in any direction. This fact is also explained as previously stated by the characteristic of the anisotropic network of the collagen fibers. Those observations are in agreement with the histological observation (Article I).

7. A smaller total amount of nitrogen is contained in the meat of *Stichopus japonicus* than in fish meat.

The total amount of nitrogen content in the meat of *Stichopus japonicus* is about 10%, contrariwisely the amount of water content is about 90 %. The total absolute amount of nitrogen content, therefore, shows as about 10% of the dried matter of the meat. On the contrary, fish meat has about 15% of the dried matter, as the amount of water content in the fish meat is 80% on the average. That is to say, the total nitrogen content of the former is smaller than that of latter. But the nitrogen distributions of the whole proteins of meat and skin parts or water-, NaCl-, and NaOH-solution soluble protein fractionated from the meat are similar with those of fish meat (Article IV). The amino acid composition of those proteins is also similar to that of fish meat though there are some differences in the amounts of amino acids (Article IV).

8. The isoelectric point of the meat of *Stichopus japonicus* is near pH 4.2, which is more to the acidic side than that of fish meat.

The isoelectric point of the meat, water soluble, NaCl solution-soluble and NaOH solution-soluble proteins are clarified to be all near pH 4.2. (Article V). This is observed to be more to the acidic side than that of fish meat. As one of the explanations of the fact is considered to be the content of a greater amount of acidic amino acids, *e.g.*, glutamic acid and aspartic acid in the meat of *Stichopus japonicus*. (Article V.) However, other possible causes must be sought for in future.

9. If the meat of *Stichopus japonicus* is repeatedly extracted with water, the aqueous extract becomes able to show flow birefringence as well as squid meat after the 3rd or 4th extraction.

If the meat is repeatedly extracted with water, the aqueous extract begins to show flow birefringence after the 3rd or 4th extraction. This phenomenon is similar to what happens with squid meat[3]. In the case of squid meat, the dissolution of a new protein component which is considered to be myosins is observed by electrophoresis, and this protein which dissolves out newly is considered to be the one which shows the flow birefringence. In the case of the meat of *Stichopus japonicus*, however, no new protein component was observed, so the substance which shows flow birefringence is considered to be some other substance. In regard to this fact, there is remarkable difference between the *Stichopus japonicus* and squid meat. This unknown substance is seemingly removed by the filtration and the filtrate shows no flow birefringence. Also, by the heating of the substance (at 90°~95°C, 5 mins.) the flow birefringence was caused to disappear, but the substance does not coagulate. The author has proposed an explanation that this substance is not a coagulable protein, but it is suspended

matters in the aqueous extract which was mechanically deformed from the collagen fibers in the connective tissue of the meat during the repeated extraction. When the meat of *Stichopus japonicus* is extracted with Weber's solution, the extract shows flow birefringence at the first extraction. This fact is considered to be due to the dissolution of salt solutions soluble proteins, *e.g.*, myosins.[8] When the residue of the first extraction with Weber's solution was washed with water until the reaction due to the presence of KCl disappeared and the residue was extracted repeatedly with water, the flow birefringence was observed again in its extract, and the intensity of flow birefringence was increased with the increasing of the number of extractions. This fact shows that the phenomenon of flow birefringence observed in the aqueous extract of the meat of *Stichopus japonicus* is due to the presence of suspended matters in its extract deformed from the network of collagen fibers in the connective tissue.

10. The meat of *Stichopus japonicus* is contracted in almost the same ratio in all directions, horizontally and vertically, by heating.

When the meat of *Stichopus japonicus* of which the radial canal was removed, is heated, the length, width and thickness of the meat contract in almost the same ratio. This fact shows that the meat consists mainly of the connective tissue of network of collagen fiber. This observation agrees with the histological observations.

11. The meat of *Stichopus japonicus* coagulates with acids ; it is difficult to gelatinize in the acid solutions of lower values of pH.

Fish meat protein or collagen generally shows the maximum swelling at near pH 2~3 and pH 9~11, and the minimum at near the isoelctric point. The swelling of fish meat is increased by the addition of salts in acidic side.[10][36] On the contrary the meat of *Stichopus japonicus* shows clearly the maximum swelling in alkali side, but in acidic stde, no remarkable swelling is observed. The swelling of the the meat of *Stichopus japonicus* is increased by the addition of salts in the alkali side but no decreasing of the swelling is observed in acidic side, in spite of the existence of the great amount of collagen[32] (Articles XII, XIII). The characteristics of this phenomenon are explained as being due to the existence of mucoprotein which is coagulated easily by acids. The existence of the mucoprotein was definitely ascertained (Article XVII).

12. The meat of *Stichopus japonicus* does not swell easily in water or in salts solutions

Stichopus japonicus meat swells with more difficulty in water and salts solutions than does fish meat. (Article XIII). This is considered to be due to the greater amount of water content in *Stichopus japonicus* meat and the smaller hydrating affinity.

13. The hydrating affinity of the meat of *Stichopus japonicus* is weak.

The hydrating affinity of the meat of *Stichopus japonicus* is weaker than that of

fish meat (Article XIV). From this fact, water in the network of collagen fibers of connective tissue is considered to be "immobilized water".

14. The meat of *Stichopus japonicus* contains mucoprotein.

The meat of *Stichopus japonicus* contains mucoprotein to the amount of 0.95% of the dried matter. The prosthetic polysaccharides of the mucoprotein in the meat are suggested to be acid mucopolysaccharide such as it in sulfuric acid and neutromucopolysaccharides containing some unit sugar composition such as hexosamine or galactose. The linked protein is ascertained by electrophoresis to consist of two components. The nitrogen distribution of the protein moiety is observed to be almost the same as that of the meat whole protein or water soluble protein (Article XVII). The presence of the mucoprotein in the meat of *Stichopus japonicus* indicates one cause for the particular phenomena in the swelling of the meat in the acidic side (Article XII). As previously explained, the meat of *Stichopus japonicus* is not true muscular tissue, but consists mainly of connective tissue composing a network of collagen fibers, in which a small amount of proteins, *e.g.*, myosin, myogen or mucoprotein, are contained ; those proteins are compoud with a great amount water. Therefore the water is present as "immobilized water." Those characteristics of the meat, *e.g.*, the smaller total amount of nitrogen content, the water content greater than that of fish meat the particularity of the presence of immobilized water, and the fact that its tissue consists mainly of the network of collagen fibers, causes the remarkable contraction by cooking and the easy deformation of the cooked meat.

SUMMARY

A few ten years ago an attempt was made to produce canned sea cucumber (*Stichopus japonicus*) but the manufacture was not continued on account of the deforming of the meat after the processing. Recently the author has succeeded to process this material as canned food after overcoming many difficulties. In the experiments on the processing, the author had difficulty to find any literature on the properties of the meat of *Stichopus japonicus*. There are only a few published studies. In sea cucumber (Holothurioidea) the edible species utilized is *Stichopus japonicus*.

The author resolved to study the meat protein of the sea cucumber (*Stichopus japonicus* SELENKA), and has obtained results as follows : (1) The edible part (body wall) of the body of *Stichopus japonicus* is histologically not true muscular tissue, but consists mainly of connective tissue composed of collage fibers, there is contained body fluid containing soluble proteins. (2) Water soluble, 0.6 Mol NaCl solution-soluble, 0.025 N NaOH solution-soluble meat protein and whole meat protein were fractionated. (3) The nitrogen distribution and the amino acid composition of the meat proteins are almost the same as those of fish meat. The amount of

protein content of the meat of *Stichopus japonicus* is only one-third of that of fish meat. (4) The isoelectric point of the fractionated proteins or the water-extract of the meat is near pH 4.2, which is more to the acidic side than that corresponding values of fish meat. (5) According to the results of electrophoresis, the water soluble and NaCl solution-soluble proteins consist of three components respectively, and NaOH solution-soluble proteins consist of four components. (6) According to the observations the solubility of the meat in water and salt solutions, the fourth water-extract of the meat by the repeated extraction shows flow birefringence as well as squid meat. The first extract of the meat with Weber's solution shows the flow birefringence. (7) The substance which shows flow birefringence in the aqueous extract of the meat is not myosins, differing from the squid meat, but it is ascertained to be suspended matters, a fibrous substance having thread-like shape. This substance is originated from the mechanical deforming from the network of collagen fibers in the connective tissue. (8) The heat contraction of the meat begins at 30°C, the greatest contractility was shown as 50~60% between 70° and 80°C. The fact that heat contractions of the length, width and thickness of the meat were almost equal offers evidence of the network construction of collagen fibers, of which tissue fibers are arranged equally length-ways and side-ways. (9) In fact, the meat of *Stichopus japonicus* contains a larger amount of gelatine-nitrogen than fish meat. That is to say, the amount of gelatine-nitrogen in the meat of *Stichopus japonicus* is 1.44~3.86% of the dried matter. The amount is 1.67% for Atka mackerel, 0.52% for carp. (10) The dehydrating phenomenon in the meat of *Stichopus japonicus* at various temperature is considered to be due mainly to the heat contraction of the collagen fibers composed of the connective tissue, and from the result of observations on the heat coagulation of 0.6 Mol NaCl solution-soluble protein, the presence of proteins corresponding to myosin and myogen is suggested. Those proteins are considered to be contained in the network of the collagen fibers of the connective tissue. (11) In the observations on the acid coagulation of the meat of *Stichopus japonicus*, the presence of mucoprotein was suggested. (12) The degree of the swelling varies according to the concentration of the solutions and valencies of the salt. In the relation between the value of pH and the degree of the swelling, the maximum swelling was observed in the alkali side. The swelling was observed to be accelerated in the more alkali side than the isoelectric point. The maximum swelling was not clearly in the acidic side. This fact is different from the fish meat. (13) The hydrating affinity of the meat and of the fractionated proteins from the meat was observed to be smaller than corresponding values in the case of fish meat. The most part of the water in the meat was suggested to be "immobilized free water" in the network of collagen fibers of the connective tissue of the meat. (14) In the hydrating affinity of the meat immersed into NaCl solutions having various concentrations, the meat immersed in 0.6 Mol NaCl solution is suggested to have the strongest affinity. (15) In the relation between the swelling and the hydrating affinity of the dried meat

swollen in water, the swelling of dried meat shows the increase of power of retaining water. (16) In the meat of *Stichopus japonicus*, the presence of mucoprotein was ascertained. The amount of mucoprotein content is 0.95% of the dried matter. The prosthetic polysaccharide such as itinsulfuric acid and neutromucopolysaccharide composed of unit sugar components such as hexosamine and galactose. The proteins linked with the polysaccharides are ascertained by electrophosresis to be two components.

The author has previously shown clearly that the meat protein of *Stichopus japonicus* is little different from the fish meat protein in the composition of amino acids, but the absolute quantity of the protein of the former is smaller than that of the latter, so the nutritive effect of the protein of the former is inferior to that of the latter. However, as stated here, the meat of *Stichopus japonicus* contains a large quantity of mucoprotein, which includes chondroitin sulfuric acid as a component.

In the studies of geriatrics which have been made much progress in recent years, the relation between the superannuating phenomena of muscle tissue and the decreasing of chondroitin sulfuric acid has been clarified. Owing to the presence of large quantities of chondroitin sulfuric acid, the fact that the Chinese have been eating sea cucumber meat as nutritive food from ancient times can be approved.

From the results above obtained, the author learned well the properties of the meat of *Stichopus japonicus*, and has devised the processing of the canned sea cucumber by immersing the meat in acetic acid solution to coagulate the protein at first and then boiling in water and drying the boiled meat. By such a processing, the author has obtained a good quality of canned sea cucumber without the deforming of the meat after the processing.

LITERATURE CITED

1) Tanikawa, E. *et al.* (1954). Bull. Fac. Fish. Hokkaido Univ. 5 (4), 338-351 ; 6 (1), 37-51.
2) Tanikawa, E. *et al.* (1954). *Ibid.* 6 (1), 52-79.
3) Okada, M. & Tada, S. (1954). Bull. Jap. Soc. Sci. Fish. 20 (30), 224-231.
4) Van Slyke, D. D. : cited from Akabori, S. (1942). Amino acids and Proteins ("Aminosan-oyobi-Tanpakushitsu") (in Japanese), 123. Kyouritsu-shuppan-sha. Tokyo.
5) Takagi, M. (1948). J. of Fisheries ("Suisangaku-zasshi") (in Japanese) 53, 1-18.
6) Kondo, K. (1936). J. Agr. Chem. Soc. Japan 12 (11), 145.
7) Fujii, T. (1954). Bull. Fac. Fish. Hokkaido Univ. 5 (3), 253-276.
8) Migita, M. (1952). Bull. Jap. Soc. Sci. Fish. 18 (10), 116-126.
9) Noguchi, E. *et al.* (1951). *Ibid.* 16 (12), 25-28.
10) Okada, M. & Tada, S. (1953). *Ibid.* 19 (3), 178-184.
11) Hardy, W. B. (1905). J. Physiol. 33, 251.
12) Ōtake, S. (1953). Monthly Report of Bureau of Fisheries, Japanese Government No. 6, 8. (in Japanese).
13) Turba, F. & Richter, M. (1942). Ber. 75. 340; cited from Mizushima, S. and Akabori, S. (1954). Chemistry of Proteins ("Tanpakushitsu-kagaku") (in Japanese) 212. Kyouritsu-shuppan-sha, Tokyo.
14) Pope, C. G. & Stevens, M. F. (1939). Biochem. J. 33, 1070.

15) Matsumoto, J. (1953). Monthly Report of Bureau of Fisheries Japanese Government No. 3, 21–23. (in Japanese).
16) Subba Rao : cited from Hamoir, G. (1950). Biochem. Soc. Sump. No. 6. The Biochem. of Fish. 15.
17) Matsumoto, J. (1953). Monthly Report of Bureau of Fisheries, Japanese Government No. 6, 5. (in Japanese).
18) Migita, M. & Matsumoto, J. (1954). Bull. Jap. Soc. Sci. Fish. 20 (7), 641–652.
19) Szent György, A. (1947). Chemistry of Muscular Contraction 4. Academic Press. New York.
20) Haurowitz, F. (1950). Chemistry and Biology of Proteins. 199. Acad. Press. New York.
21) Migita, M. & Tanaka, T. (1953). Monthly Report of Bureau of Fisheries, Japanese Government No. 3, 18. (in Japanese).
22) Niiyama, E. (1954). unpublished.
23) Takahashi, T. (1953). Bull. Jap. Soc. Sci. Fish. 19 (4), 603–610.
24) Takahashi, T. (1943). *Ibid.* 12 (1), 35–41.
25) Mitchell, H. H. *et al.* (1926–27). J. Biol. Chem. 71, 379.
26) Kashiwada, K. & Kakimoto, D. (1952). Bull. Jap. Soc. Sci. Fish. 18 (5), 17–21.
27) Simidu, W. (1943). *Ibid.* 12 (2), 73–81 ; 12 (3), 103–106.
28) Takagi, M. (1950). Bull. Fac. Fish. Hokkaido Univ. 1 (1), 28–34.
29) Simidu, W. & Takeda, K. (1951). Bull. Jap. Soc. Sci. Fish. 17 (5), 18–24.
30) Tanikawa, E. (1943). Salted Food ("Enzo-Shokuhin" in Japanese) 64. Seikatsu-sha. Tokyo.
31) Fürth, O. V. (1895). Arch. f. exper. Pharm. u. Path. 36. 231.
32) Takahashi, T. (1954). Bull. Jap. Soc. Sci. Fish. 19 (12), 1200–1218.
33) Lloyd, D. J. & Marriott, R. H. (1933). Trans. Faraday Soc. 29, 1228.
34) Sasaki, T. (1948). J. Jap. Chem. ("Kagaku-no-Ryoeki") (in Japanese) 2 (8); 2 (9); 3 (2).
35) Mizushima, S. (1949). Physical Chemistry ("Butsuri-kagaku") (in Japanese) 4, 1021. Kyouritsu-shuppan-sha. Tokyo.
36) Tarr. H. L. A. (1942). J. Fish. Res. Bd. Canada. 5, 411.
37) Haurowitz, F. (1950). Chemistry and Biology of Proteins 93. Acad. Press. New York.
38) Sponsler, O. L. *et al.* (1940). J. Phys. Chem. 44, 996.
39) Akiba, M. (1951). Bull. Fac. Fish. Hokkaido Univ. 1 (3–4), 156–196.
40) Lewis, G. N. and Randall, M. (1923). Thermodynamics XXIII : 284.
41) Brunauer, S. *et al.* (1938). J. Am. Chem. Soc. 60, 309.
42) Bull. H. B. (1944). *Ibid.* 66, 1499–1507.
43) Higashi, K. & Nukazawa, K. (1948). Chemical Researches ("Kagakuno-Kenkyu" in Japanese) 1. Biochemistry 23.
44) Akiba, M. (1951). Bull. Fac. Fish. Hokkaido Univ. 2 (3), 176–213.
45) Tanikawa, E. & Onodera, H. (1953). "Aomori-ken Suisan Shigen Chosahokoku" (in Japanese) 3, 77.
46) Takahashi, T. (1953). Bull. Jap. Soc. Sci. Fish. 19 (5), 693–696.
47) Akiba, M. (1952). Bull. Fac. Fish. Hokkaido Univ. 2 (4), 239–261.
48) Wörner, C. (1889). Skand. Arch. f. Physiol. 1, 210.
49) Hisamura, H. (1938). J. Biochem. Japan 28, 217.
50) Hiruse, S. & Araki, C. (1951). Chemical Researches ("Kagaku-no-Kenkyu" in Japanese) 5 (7), 28–35.
51) Masamune, H. *et al.* (1953). Tohoku. J. Exp. Med. 58 (3–4), 381–398.
52) Partridge, S. M. (1946). Nature 158 (4008), 270.
53) Partridge, S. M. (1948). Biochem. J. 42, 238, 251.
54) Tanabe, Y. (1938). J. Biochem. Japan 27, 251–256.
55) Koganei, R. (1933). Micro method of quantitative biochemical determination ("Seikagaku-teki Biryo-teiryo-ho" in Japanese) 341.

56) Gale, E. G. (1937). Biochem. J. 31, 234.
57) Suzuki, M. (1938). J. Biochem. Japan 27 (2), 367.
58) Masamune, H. & Nagazumi. Y. (1937). *Ibid.* 26, 223.
59) Blix, G. & Snellmann, O. (1945). Arkiv. Kemi. Mineral. Geol. Alg. 32.
60) Haurowitz, F. (1950). Chemistry and Biology of Proteins. 181. Acad. Press. New York.

昭和 30 年 7 月 20 日　印　刷
昭和 30 年 8 月 1 日　発　行

北 海 道 大 學 水 產 学 部
函 館 市 港 町 253 番 地

印 刷 所　笹気出版印刷株式会社
仙 台 市 堤 通 27 番 地

MEMOIRS

OF THE

FACULTY OF FISHERIES

HOKKAIDO UNIVERSITY

Vol. 1, No. 1 (March, 1953)

MOTODA, S. OBSERVATIONS ON DIURNAL MIGRATION OF PLANKTON CRUSTACEANS IN LAKES SHIKOTSU, HOKKAIDO, AND TSUGARUJUNI, AOMORI, AND SOME EXPERIMENTS ON PHOTO- AND GEOTROPISM

Vol. 2, No. 1 (December, 1954)

TOKIDA, J. THE MARINE ALGAE OF SOUTHERN SAGHALIEN

Vol. 3, No. 1 (August, 1955)

TANIKAWA, E. STUDIES ON THE PROTEINS OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)